THE EFFECT OF LOW DOSE GAMMA RADIATION ON THE QUALITY OF INTERMEDIARY MOISTURE PET FOOD

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

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DECLARATION

I hereby declare that the thesis entitled **"THE EFFECT OF LOW DOSE GAMMA RADIATION ON THE QUALITY OF INTERMEDIARY MOISTURE PET FOOD"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Certified that the thesis, entitled **"THE EFFECT OF LOW DOSE GAMMA RADIATION ON THE QUALITY OF INTERMEDIARY MOISTURE PET FOOD"** is a record of research work done independently by **RANA RAJ. V. R.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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INTRODUCTION

The first commercially prepared dog food was a biscuit product introduced in England during 1860's. After a long gap of nearly 100 years, in 1960's, more varieties of pet foods like canned products and new semi moist or intermediary moist products were introduced. Presently the available pet food for cats and dogs in the local market can be classified on the basis of their moisture content and method of preservation into three basic groups, *viz.*, dry, canned and semi moist/ intermediate moisture pet food. The increased adoption of pets has provided a momentum for the production of good quality pet foods (Mermelstein, 1998) which has got a high market value (Downing, 1999).

The most popularly available pet foods in the market are of low moisture content or dry with a moisture content of about 5 per cent. They have got higher shelf life than other foods. The main disadvantages of these foods are that they are hard and brittle. Dry foods are less palatable than other types and only dry ingredients can be used in their formulation. Harsh drying is required if wet ingredients are used, which can reduce the nutrient content and digestibility of many of the ingredients. Canned (high moisture) pet food (75 to 85 per cent moisture) makes a very important contribution to the diet of cats and dogs (Rainbird, 1988). High quality nutritionally balanced canned products are usually higher in animal proteins and animal fat but lower in carbohydrates than dry or semi moist products (Kelly, 1996). The third variety is semi-moist / intermediary moisture pet foods which are of moisture content about 25 to 30 per cent. Most of the intermediary moisture pet foods are quite palatable. A major advantage of intermediary moisture pet food is that more

ingredients, including fresh animal tissues can be used in their formulation. It can effectively utilize the slaughter house waste materials. The major disadvantage of this type of intermediary moist food is that it becomes more vulnerable to bacterial and fungal spoilage due to its increased water activity. So shelf life of these products will be low. To overcome these defect some type of preservation techniques should be adopted.

The development of food preservation processes has been driven by the need to extend the shelf-life of foods whilst maintaining their safety. Preservation methods that have long been accepted by consumers, however, frequently have associated disadvantages, in particular adverse changes in organoleptic characteristics and loss of nutrients. Heat processing can cause significant deterioration in the sensory properties of food. Even mild heat treatments cause substantial flavor changes in products. Freezing causes severe textural deterioration in foods such as strawberries. Even more modern minimal processing techniques, such as modified atmosphere packing and sous-vide cooking, each of which gives relatively small changes in sensory quality, add to product cost and can carry microbiological hazards.

Irradiation of pet foods produces organoleptic changes that are much less serious than those quoted above, and carries no serious side-effects when a controlled process is applied to appropriate foods. Considering the advantages of irradiation as a method of food preservation, Food and Drug Administration, (FDA) 1997 approved an irradiation dose of 25 kGy (max.) for animal feed and pet food for decontamination (FDA, 1997). But a low dose irradiation, radurization, is enough to extend the shelf life of the products. It is currently used in the United States for feeds for research and lab animals. Irradiation can eliminate most of the harmful microorganisms in pet foods and pet treats making them safer for the pets.

The present study is aimed at producing a shelf stable intermediary moisture pet food using slaughter house byproducts and to assess the effect of irradiation on the keeping quality of pet food having different levels of moisture and also to assess the acceptability of these foods by the dogs.

Review of Literature

REVIEW OF LITERATURE

Cats and dogs are the important pets reared in our country. Animal owners are running behind for quality pet foods. Branded items are available only in super markets of cities. The cost of these branded pet foods available varies around Rs 100/- per kilogram. These foods are having an expiry date of nearly one year. In order to achieve this target, pet foods are dried / ovened to a moisture level of less than 5.0 per cent. This type of hurdling processes definitely leads to difficulty in chewing and also loss of certain nutrients.

2.1. INGREDIENTS OF DOG BISCUIT

Mongeau and Brassard (1985) conducted a study on dietary fibre and faecal characteristics in dogs and concluded their study that the changes in faecal density observed following a modification in the level or particle size of dietary bran which indicated that bulk should be included among rest of the ingredients for adequate characterization of faeces from the view point of luxation.

Fahey *et al.* (1992) studied on the dietary fibre in dogs and found that at levels of 7.5 per cent or below the fibre source had no adverse effect on nutrient intake, nutrient digestibility or metabolizable energy values. So they suggested that high fibre products can be used in meat based dog diets at concentrations of 7.5 per cent or less of diet dry matter.

The slaughterhouse byproducts are good sources of protein, minerals and vitamins for pets but they will be contaminated with high number of microorganisms. Therefore, it is imperative that these byproducts be adequately processed to render them safe before feeding them to animals. (Urlings *et al.*, 1993)

In a study conducted on the use of raw meat based diet or a dry kibble diet for sand cats, it was revealed that the raw meat based diet was almost 15 per cent more digestible than the kibble diet. (Crissey *et al.*, 1997)

Animal byproducts in the diets of dogs are good sources of digestible nutrients. The protein and fat components of diets containing these products are highly digestible at the ileum. Ileal amino acid digestibility is also high in case of proteins of animal origin. (Murray *et al.*, 1997).

Opitz *et al.* (1998) studied various methods of fibre analysis in pet food and their study indicated that cellulose is the major source of fibre in pet foods.

Dobenecker and Kienzle (1998) found that the digestibility of organic matter in the fibre supplemented diets was reduced to between 45 and 63 per cent although the non fibre compounds of the experimental diets have shown high digestibility of about 90 per cent. So they stated that more fibre should be added in the diet for obese dogs and cats.

Earle *et al.* (1998) studied the effect fibre in the digestibility of pet food and found that high dietary fibre can reduce digestibility of organic matter in many species including dogs and cats.

Regland *et al.* (1998) stated that animal byproducts generated from animal slaughter have traditionally served as protein and mineral supplements for cats and dogs and had been evaluated as a rich source of proteins, minerals and many vitamins for all the pet animals.

Murray *et al.* (1999) evaluated some high starch flours as ingredients in canine diet and found that ileal and total digestibilities were different among various starch diets, but the starch components of all diets were almost completely digested (> 99 per cent). They also found that dogs exhibited the highest dry matter digestibility at ileum (81.2 per cent) when fed the wheat flour.

Revera *et al.* (2000) concluded in their study on high moisture pet food that the water retention capacity of meat byproducts were not improved by non meat ingredients like salt, phosphates or sodium hydroxide. So these non meat ingredients are not important to meat ingredient functionality of pet food systems with high levels of meat by products.

Yamaka *et al.* (2003) evaluated low ash poultry meal as a protein source in canine foods and concluded their findings that a high quality protein source should contain all essential amino acids in proper amounts and be readily bioavailable for dogs and the protein from animal origin will serve the requirements. They also stated that poultry meal in dietary inclusions of 10 to 32.5 per cent of the food can be an excellent source of protein for dogs.

Ahlstrom *et al.* (2004) studied the fatty acid composition in commercial dog food and they stated that dietary fat from animal origin supplies essential fatty acids and were crucial for carrying many fat soluble vitamins. They also stated that in dry extruded dog food, fat of either animal or vegetable origin or a mixture of both can be used.

Gajda *et al.* (2005) stated that cereal grains are used in extruded pet foods because of their high nutritive value and their relatively low cost. They also reported that extrusion enhances the value of the cereal grains by cooking the starch components, thereby increasing the digestibility of the complete diet.

2.2. PROXIMATE COMPOSITION

Miller (1985) conducted a study on extrusion of dry pet foods and found out that extrusion at low moisture is responsible for the reduction in plasticity and elasticity of extrudate. They also stated that low moisture extrusion decreased product uniformity up to 45 per cent and product shape roundness up to 70 per cent.

Lomauro *et al.* (1985) studied the moisture transfer of dry and semi moist foods and they stated that the amount of water present at any location in the food affects characteristics such as drying time and shelf life.

National Research Council (1985) divided dog food into three basic types *i.e,* dry, semi moist and canned dog foods depending on the moisture content. They also insisted that the moisture content of semi moist dog food as 25 to 30 percent.

In a study conducted by Katta *et al.,* (1991) found that chicken carcass irradiated at various dose levels ranging from zero to 3.0 kGy using gamma radiation and stored in refrigerator conditions were shown no variation in their fatty acid profile.

Diehl (1995) observed no significant changes in amino acid composition when bone meal was irradiated even up to 50.0 kGy.

Earle *et al.* (1998) found a significant negative relationship between crude fibre in the dry matter and the apparent digestibility of either organic matter or energy.

Wheeler *et al.* (1999) conducted a study on the proximate composition of ground beef patties and they found that there were no effects of irradiation dose or patty location on percentages of fat or moisture of patties. 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.

Marathe *et al.* (2002) found that irradiation (1.0 kGy) of prepacked whole wheat flour had shown no adverse effect of irradiation and storage up to 6 months on total proteins, fat, carbohydrates and vitamin content.

In a study conducted by DeRouchey *et al.,* (2003b) stated that there was no significant effect on total amino acid, true digestibility, biological value or

net protein utilization of a complete rat diet when irradiated at 25.0 and 100.0 kGy.

Al-Bachir (2005) studied on irradiation of spices, packaging material and luncheon meat to improve storage life of the end product and observed that there was no significant difference in moisture, protein and fat contents of the products as a result of irradiation during a period of 12 months in refrigerated storage.

2.3. RADIATION OF FOOD

2.3.1. Antimicrobial Action

The continuous decrease of microbial population in irradiated samples on storage was postulated to the fact that the surviving cells will be having varying degrees of injury due to irradiation and depending on the extend of injury, many may die off in course of time. (Singh *et al.*, 1991)

Katta *et al.* (1991) studied the effect of gamma irradiation in whole chicken carcasses on bacterial loads and fatty acids and their results indicated that more than 99 per cent of the microbial load in the whole carcass was eliminated at doses ranging from 1.5 to 2.0 kGy.

In a study conducted by Patterson (1996) on irradiation in combination with other preservation techniques found that irradiation followed by heat treatment had a synergistic effect on the destruction of bacterial spores and vegetative cells.

The effectiveness of irradiation against pathogens is mainly due to hydrogen peroxide production that results from the generation of free radicals during irradiation. Hydrogen peroxide acts as a potent anti microbial and can eventually result in the production of long lived hypochlorite, which is very toxic to pathogens. (Lewis *et al.*, 2002)

Lee (2004) explained the action of irradiation that it will remove electrons from water and produce free radicals, which attack the DNA of the microorganisms. He also stated that an absorbed radiation dose of 0.1 kGy resulted in about 2.8 per cent damage of the DNA which was mainly from indirect ionization of water, rather than direct DNA hits.

Zhu *et al.* (2004a) stated that the bactericidal action of ionizing irradiation is largely linked to damage of bacterial DNA from the production of free radicals during the irradiation process.

2.3.2. Food Safety

The use of ionizing radiation as a method of food preservation has been studied since the early 1940's. The major applications of food irradiation include sterilization, pasteurization, disinfection, disinfestations, shelf life extension and product development. (Nagai and Moy, 1985)

Dempster (1985) stated that low dose irradiation or radurization eliminates most of the parasites in pork and very importantly, salmonella organisms in poultry and red meat. So radurization will increase the shelf life of poultry meat, red meat and meat products significantly. Therefore, irradiation has an important role to play in public health protection.

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

Monk *et al.* (1995) stated that D_{10} values of 2.0 and 2.4 kGy were determined for Hepatitis A virus and Rota virus, respectively. They also observed a 100 per cent reduction of Poliovirus in fish fillets after an irradiation dose of 6.0 kGy

Medium dose irradiation (1.0 to 10.0 kGy) has been approved by the United States Department of Agriculture (USDA) for raw meat and poultry for decontamination. (Olson, 1998)

Marathe *et al.* (2002) studied the gamma irradiation of whole wheat flour and concluded their study that gamma irradiation at 0.25 kGy was sufficient to extent the shelf life of *atta* up to 6 months with out any significant changes in the nutritional and functional attributes. They also found that *chapathies* made from irradiated *atta* (0.25 kGy) were preferred even after 6 months storage, compared with the control.

Kuttinarayanan *et al.* (2006) stated that the treatment of meat with ionizing radiation is an effective method to reduce or 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.

2.3.3. Radiation Processing of Animal Foods

Irradiation was shown to reduce bacterial concentrations in protein meals of animal origin that were fed in commercial diets of livestock without damaging protein quality, unlike that caused by heat sterilization.(Carpenter, 1963).

Eggum (1979) concluded that irradiation up to 50.0 kGy did not diminish the nutritional value of fish meal to any significant extent and should be given preference over heat treatment, which reduces the nutritional value.

In 1997 Food and Drug Administration (FDA) approved irradiation of pet food and animal feed to a maximum dose of 25.0 kGy for controlling Salmonella organisms. (FDA, 1997)

DeRouchey *et al.* (2003a) evaluated the effects of blood meal pH and irradiation on nursery pig performance and found that the nursery pig

performance was improved when pigs were fed on irradiated blood meal compared with blood meal in regular form.

In another study on irradiation of individual feed ingredients on nursery pig performance, DeRouchey *et al.* (2003b) showed that irradiation of individual ingredients or whole diet did not negatively impact pig performance compared with non irradiated diets, and therefore would be considered a safe processing technique for feed ingredients.

Grolichova *et al.* (2004) confirmed in their study that ionizing radiation could be used for sterilization of diets for laboratory animals, pathogen free animals and animals used for the health control programmes. They concluded that feeding diets treated with ionizing radiation reduces the risk of contamination originating in animal herd.

2.4. THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)

Hampson *et al.* (1996) stated that the presence of malonaldehyde in meat is indicative of oxidative damage of unsaturated lipids present in it and this malonaldehyde content can be determined by thiobarbituric acid reactive substance (TBARS) measurement.

More pronounced oxidative rancidity and less stable display color were noted for samples irradiated in aerobic packaging. Irradiation source had varying but limited effects on color and rancidity. Optimum packaging conditions can control color and rancidity changes in boneless pork chops, thereby enabling irradiation to be a useful intervention technology. (Luchsinger *et al.*, 1996)

Irradiation causes accelerated lipid oxidation in raw meat during subsequent storage. But oxygen exposure is a more important factor than irradiation in catalyzing lipid oxidation of raw meat patties during storage. (Ahn *et al.*, 1998)

Ahn *et al.* (2000a) observed that the TBARS values were increasing sharply during refrigerated storage in aerobic packaging, but the effect of irradiation was not found at 2 weeks of storage. So they concluded that the storage condition or oxygen availability was more important for the development of lipid oxidation than irradiation.

Ahn *et al,* (2000b) showed that irradiated muscle strips produced more TBARS values than nonirradiated only in aerobic packaging during storage. So they concluded that irradiation had no effect on the production of volatiles related to lipid oxidation.

Du *et al.* (2001) studied the effect of irradiation on the TBARS values of vacuum and aerobic packaged cooked meat. They did not find any significant difference on day zero. Nam *et al.* (2001) reported that irradiation and packaging influenced the TBARS values of turkey and pork, but not of beef in 7 days of storage time.

Nam and Ahn (2002) noticed that the free radicals generated by irradiation accelerated lipid oxidation only when irradiated meat samples were aerobically stored. They opined that the presence of oxygen was the most critical factor influencing lipid oxidation in meat.

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 included alkenes, aldehydes and sulphur compounds.

Gomes *et al.* (2003) stated that the differences in off odours between irradiated and nonirradiated raw chicken was not correlated to lipid oxidation and concluded that sulphur compounds like dimethyl trisulfide were the main volatile components responsible for irradiation odour.

In a study on effect of irradiation on properties of cured ham, Houser *et al.* (2003) found that all irradiation treatments had significantly higher TBARS values than the non irradiated control.

Irradiation promoted lipid oxidation of turkey meat, but the increased lipid oxidation was problematic only when the irradiated meat was aerobically packaged and stored. (Nam and Ahn, 2003).

Lee and Ahn (2003) found that TBARS values of irradiated emulsion samples immediately after irradiation were lower than those of non irradiated samples. After 5 days of storage at 4°C irradiated samples developed higher TBARS values than non irradiated emulsions.

Zhu *et al.* (2003) observed that irradiated turkey ham had significantly higher TBARS values than nonirradiated control on the day of preparation, but this difference disappeared after 7 and 14 days of storage.

Irradiated rabbit meat produced significantly higher TBARS than non irradiated samples and the amounts of TBARS showed positive correlations with the applied dose and storage time. (Badr, 2004)

Ohene-Adjei *et al.* (2004) observed significant increase in TBARS compared to the control for aerobically packaged pork irradiated at 4.5 kGy and stated that irradiation dose had no significant effect on TBARS of vacuum packaged pork.

Zhu *et al.* (2004b) stated that irradiation and storage increased TBARS values, which could be because of the presence of residual oxygen or oxygen permeable packaging material during storage.

Kanatt *et al.* (2005) found that non irradiated control samples showed lower TBARS values than irradiated samples and concluded that the TBARS values were dose dependent.

Nortje *et al.* (2005) observed that TBARS values of irradiated biltong differed only slightly and insignificantly from that of the non irradiated samples.

2.5. MICROBIOLOGICAL LOAD

2.5.1. Aerobic Plate Count

Sharma *et al.* (1989) conducted a study on the microbiological quality of gamma irradiated Indian spices and concluded that standard plate count was reduced to zero to100 cfu/g for spices irradiated at 10.0 kGy dose of gamma rays.

A study on buffalo meat conducted by Naik *et al.* (1993) revealed that there was a marked reduction in total count by 2 to 3 log cycles due to radiation treatment. They also stated that irradiated meat was completely free of *Pseudomonas* spp. and *Enterobacteriaceeae* throughout storage.

Rao *et al.* (1994) conducted a study on the shelf life of gamma irradiated *rawa* and found that radiation treatment produced a marked reduction in total count. They concluded that gamma irradiation at 0.25 kGy effectively extended the shelf-life of *rawa*, packed in pouches made from low density polyethylene laminate, for six months.

In a study on the effect of irradiation and chiller storage on the microbiological and sensory quality of a ready meal, Mcateer *et al.* (1995) showed that irradiation of 2.0 and 3.0 kGy reduced the number of microorganisms in the meal to less than100 cfu/g initially and significant microbial growth did not occur during storage. They also stated that a combination of low dose irradiation and chiller storage effectively controlled the microbial growth.

Gursel and Gurakan (1997) found that irradiation at 2.5 kGy prior to refrigeration is an efficient way for the preservation of meat products contaminated with *Listeria monocytogenes* and observed that irradiation dose of

2.5 kGy was sufficient to reduce *Listeria monocytogenes* count from 10^4 to zero on the day of preparation.

Gamage *et al.* (1997) showed that microbial counts on ground beef irradiated at 2.2 to 2.4 kGy dose did not exceed 7.5 $\log_{10}$ cfu/g during 34 days of storage, while this level was reached in non irradiated samples by day 13.

The meat products were subjected to gamma irradiation of 2.5 kGy under cryogenic condition by Alur *et al.* (1998) and observed that the counts of mesophilic aerobes and *Staphylococcus* were reduced by 3 to 4 log cycles compared with the control samples.

Murano *et al.* (1998) observed 3 $\log_{10}$ reductions in total aerobic counts immediately after irradiation and there was no difference in lipid oxidation with in the first weak of storage in case of ground beef.

Shelf life of ground beef patties treated by gamma radiation was studied by Roberts and Weese (1998) and observed that beef patties treated at 1.0 and 3.0 kGy reached spoilage aerobic plate count levels (>10^7 cfu/g) by 14 days and 21 days respectively, whereas patties treated at 5.0 kGy did not spoil until 42 days. The non irradiated control samples for both batches of ground beef spoiled within 7 days.

Lewis *et al.* (2002) found that an electron beam irradiation dose as low as 1.0 kGy result in a 2 to 3 log cycle reduction in the total number of aerobic organisms.

DeRouchey *et al.* (2003a) evaluated the effects of blood meal pH and irradiation on nursery pig performance and they found that the liquid plasma had initial bacterial concentrations ranging from 1.5×10^6 to 3.2×10^6 cfu/cm^3 and after irradiation bacterial counts linearly decreased from 8.5×10^1 to zero cfu/cm^3 as the dosage was increased.

Badr (2004) reported that irradiation of meat at 1.5 and 3.0 kGy significantly reduced the counts of aerobic mesophilic bacteria, psychrophilic bacteria, yeast and mould and prolonged the refrigerated shelf life of samples to 12 and 21 days respectively compared to 6 days for non irradiated controls.

Kuttinarayanan *et al.* (2005) found that the initial microbiological load with respect to total plate count reduced by 95 per cent on the day of preparation by irradiation of turkey breast at 1.0 kGy.

2.5.2 Coliforms and Escherichia coli (E. coli)

Sharma *et al.* (1989) concluded in their study that an irradiation dose of 10.0 kGy is sufficient to eliminate the total *E.coli* organisms in spices.

Thayer and Boyd (1993) concluded that *E. coli* 0157:H7 was very sensitive to gamma radiation at dosages within the range of 1.5 to 3.0 kGy, indicating that *E.coli* could be very effectively controlled in meat and meat products. They also showed that 90 per cent of viable *E. coli* in chicken meat was eliminated by doses of 0.27 kGy at $5^{O}C$ and 0.42 kGy at $-5^{O}C$.

Taxue (2001) conducted a study on the public health aspect of irradiated food materials and reported that irradiation had destroyed E. *coli* O157:H7, *Campylobacter*, *Salmonella*, *Listeria* and *Toxoplasma* organisms in food materials.

Chirinos *et al.* (2002) reported a dose of 1.08 kGy would be sufficient to reduce *E. coli* O157:H7 contamination by 4 log cycles, with out affecting the sensory attributes of hamburgers.

Lewis *et al.* (2002) concluded that coliforms can be eliminated completely from poultry meat by an irradiation dose of 1.0 kGy.

Kanatt *et al.* (2005) reported that the meat samples which were irradiated at 2.5 kGy were free from fecal coliforms compared to non irradiated samples.

2.5.3. Salmonella

Loken *et al.* (1968) revealed that Salmonella was most frequently found in the protein feed supplements produced by rendering plants and Tompkin and Kueper (1973) stated that the most frequent contaminant of rendered animal by-products was Salmonella.

Tarkowski *et al.* (1984) conducted low dose gamma irradiation of raw meat and they concluded that doses as low as 1.0 kGy were effective in reducing *Salmonella* by approximately 1.6 to 2.7 log cycles in filet American and 1.3 to 1.8 log cycles in ground meat.

Katusin-Razem *et al.* (1992) stated that an irradiation dose of 2.5 kGy was adequate for of 10^3 reduction of for Salmonella from egg products.

Alur *et al.* (1998) revealed that a radiation dose of 2.5 kGy was sufficient in eliminating Salmonella from the pork products without causing any adverse effect on the texture, odour, flavour and pigments of the products.

Farkas (1998) stated that radiation treatments at a dose of 3.0 to 5.0 kGy for frozen poultry and 1.5 to 2.5 kGy for chilled poultry were effective for reducing the most resistant Salmonella by about 3 log cycles.

Pitout *et al.* (2003) found that handling of pet treats containing animal products, which are available in pet shops and retail stores, could play a role in the increasing prevalence of AmpC- producing *Salmonella entrica* serotype

A study conducted by Lee (2004) found that at irradiation by 1.0 to 3.0 kGy reduced the spoilage microorganisms present in packaged foods and

3.0 kGy had completely destroyed food poisoning organisms such as Salmonella, Campylobacter and Listeria.

Zhu *et al.* (2004a) stated that irradiation is the most effective way to eliminate pathogens, including *Listeria monócytogenes*, Salmonella and *Yersinia enterocolotica* .

2.5.4. Staphylococcus

A study on irradiated ground beef conducted by Monk *et al.* (1995) revealed that The D_{10} values of *Listeria monocytogenes* and *Staphylococcus aureus* were ranged from 0.507 to 0.610 kGy and 0.435 to 0.453 kGy respectively. They also stated that neither the fat content of beef nor the temperature during irradiation treatment influenced inactivation rates of the two pathogens.

Decontamination of food by ionizing radiation is safe, efficient, environmentally clean and energy efficient process. Radiation treatment at doses of 2.0 to 7.0 kGy, depending up on the condition of irradiation and the food, could effectively eliminate potentially pathogenic non spore forming bacteria including both long-time recognized pathogens such as *Salmonella* and *Staphylococcus aureus* as well as emerging or new pathogens such as *campylobacter, Listeria monocytogenes* or *Escherichia coli* O157:H7 from suspected food products without affecting sensory, nutritional and technical qualities (Farkas, 1998).

Lamb *et al.* (2002) reported that refrigerated sandwiches irradiated at 5.9 kGy showed no *Staphylococcus aureus* growth on the day of preparation. Sandwiches irradiated with 5.9 kGy showed 6.18 log reduction in *Staphylococcus aureus* after 13 days and non irradiated sandwiches showed a 0.53 log increase in *Staphylococcus aureus* after 39 days.

A study conducted by Kuttinarayanan *et al.* (2006) observed that there was 94 to 98 per cent reduction with respect to aerobic plate count, staphylococcal count and yeast and mould count in beef fry irradiated at 2.0 kGy.

2.5.5. Clostridium

Kempe *et al.* (1957) stated that ground beef packed in No.1 picnic tin cans and inoculated with *Clostridium botulinum* 213B spores was sterilized by combined irradiation and heat processing.

Midura *et al.* (1965) studied the effect of gamma irradiation in *Clostridium perfringens* (*C. perfringens*) spores and they found that D values for *C. perfringens* type A spores lies between 0.23 to 0.28 Mrad in phosphate buffer while the same organism shown D value of 0.21 to 0.48 Mrad in Robertson's cooked meat medium.

Gombas and Gomez (1978) stated that heat activated spores were given a lethal dose gamma radiation of 0.3 to 0.7 Mrad and recorded 40 to 99 per cent reduction in colony-forming units (cfu) in case of *C. perfringens.* They also observed that high heat followed by gamma irradiation, was found to have an additive effect on the rate of *C. perfringens* spore inactivation.

Smith and Pillai (2004) stated that irradiation is the best method to decontaminate food materials as it destroyed campylobacter, *C. Perfringens, E. coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* at low irradiation doses.

Twenty five commercial raw diets for dogs and cats were bacteriologically evaluated by Weese *et al.* (2005) and reported that all the samples were contaminated with varying levels of coliforms, *E. coli*, Salmonella, Clostridium and Staphylococcus organisms.

2.5.6. Yeast and mould

Narvaiz *et al.* (1988) compared the effects of heat and radiation on *Aspergillus parasiticus* and the results obtained showed that heated or irradiated samples have decreased aflatoxin levels compared to control nontreated samples, and the combined treatment reduced the aflatoxin level below the detection limit of less than 30 ppb.

Monk *et al.* (1995) reported that yeast populations on chicken breast were reduced from 5.0×10^2 cfu/g to 3.2×10^1 cfu/g upon treatment with 2.5 kGy of irradiation. They also stated that *Sporobolmyces roseus* exhibited least resistance and *Trichosporon* and *Candida.* shown maximum resistance towards gamma radiation.

A study conducted by Abu-Tarboush *et al.* (1997) showed that yeasts of the genera candida, saccharomyces and alternaria started to grow on day 12 in chicken treated with less than 5.0 kGy, but not in the samples treated with more than 5.0 kGy

Nieto-Sandoval *et al.* (2000) observed that moulds, yeasts, and sulfite reducing clostridia were the most resistant species, although a 10.0 kGy dose of irradiation leads to optimum sanitation in red paprika.

A study about the effect of gamma irradiation on aflatoxin B1 levels and fungal infection in peanut samples conducted by Prado *et al.* (2003) revealed that irradiation by of 10.0 KGy completely inhibited the growth of moulds. They also suggested that the decontamination of moulds by irradiation, before production of aflatoxin B1, is the most acceptable method of preservation.

Rizk and Moussa (2003) showed Gamma irradiation at 4.0 kGy eliminated most of the fungal organisms like Alternaria, Fusarium and Epicococcum species but 12.0 kGy was required to kill bacillus species in sugar beet seeds.

Balamatsia *et al.* (2006) studied the effect of low dose irradiation on the microbiological characters of chicken meat stored aerobically at $4^{O}C$ and they found that pseudomonas, enterobacteriaceae, yeast and moulds were highly sensitive to gamma irradiation and were completely eliminated at 2.0 kGy.

Kuttinarayanan *et al.* (2006) observed a 97 per cent reduction with respect to yeast and mould count in minced beef by irradiation at 2.0 kGy.

2.6. PALATABILITY

Zuo *et al.* (1996) stated that a dog food that contains high quality animal products generally will have higher digestibility than plant based food.

In a study Hendriks *et al.* (1999) concluded that over heat processing above the minimum time required for sterilization of the canned moist diets for cats resulted in changes in the digestibility of amino acids and the majority of amino acids showed a decrease in digestibility as heat processing time increased.

Wheeler *et al.* (1999) observed no significant difference in sensory qualities between irradiated and non irradiated meat patties until after 5 weeks of storage in frozen condition.

Kastenmayer *et al.* (2002) conducted a study on the mineral and trace element absorption in adult dogs and reported that apparent absorption of calcium, iron, and zinc from commercial dry food by adult dog is low compared to the absorption from the moist pet food.

Ahlstrom *et al.* (2004) reported that fat is the most energy concentrated nutrient and is important for palatability and texture in pet food. The fat of animal origin contains more essential fatty acids than fat of vegetable origin.

In a palatability study Araujo and Milgram (2004) had stated that palatability is the measure of subjective food preference and depends on taste,

texture and odour. So as the palatability increased, the administration of the substance will become easier and concluded that semi moist pet food was preferred more by dogs compared to dry pet foods.

Krogdahl *et al.* (2004) studied nutrient digestibility of commercial dog foods and concluded that the digestibility of main nutrients varies significantly among commercial dry dog foods. They demonstrated that there was no difference in digestibility of nutrients between high price and low price dog foods offered in the Norwegian market.

Spears *et al.* (2004) evaluated stabilized rice bran as an ingredient in dry extruded dog diets and implied that addition of stabilized rice bran to dry dog food at the 12 per cent concentration shown to be more palatable than defattened rice bran.

Materials and Methods

MATERIALS AND METHODS

In the present study the effect of low dose gamma radiation on the quality of intermediary moisture pet food was assessed. The most popular and fast moving dog biscuits were prepared using meat cum bone meal, plain flour (maida), blood, rendered fat, eggs, wheat bran and black gram flour. The mix was baked at different time-temperature combinations in order to prepare biscuits with different moisture levels. Biscuits at each moisture level were subjected to different doses of gamma radiation. The various quality parameters, *viz.*, proximate composition, microbiological quality, development of rancidity and palatability attributes of irradiated and non irradiated biscuits were assessed.

3.1. PREPARATION OF DOG BISCUIT

Dog biscuit was prepared using the various ingredients and the recipe is shown in the Table 1 and the flow chart for preparation is given in the Figure1. All ingredients were mixed thoroughly and dough was prepared. This dough was then molded into rectangular biscuits in such a way that each biscuit after baking should attain a weight of 6 to 8g. These biscuits were then baked in an oven at a temperature of 200° C for varying time to attain the desired moisture level. The time temperature combinations in baking to attain various moisture levels are given in the Table 2. The prepared biscuits were grouped in to four categories based on the moisture content *i.e,* to 5, 10, 15 and 25 per cent. After baking the biscuits were cooled to room temperature and each category was packed aerobically in High Density Polyethylene (HDPE) packets of 200g. Except five per cent moisture sample, biscuits of all other moisture levels (10, 15 and 25 per cent) were divided in to 66 packets each. These were used to study the effect gamma irradiation at dose levels of 2.0, 3.0, 4.0, 6.0 and 8.0 kGy.

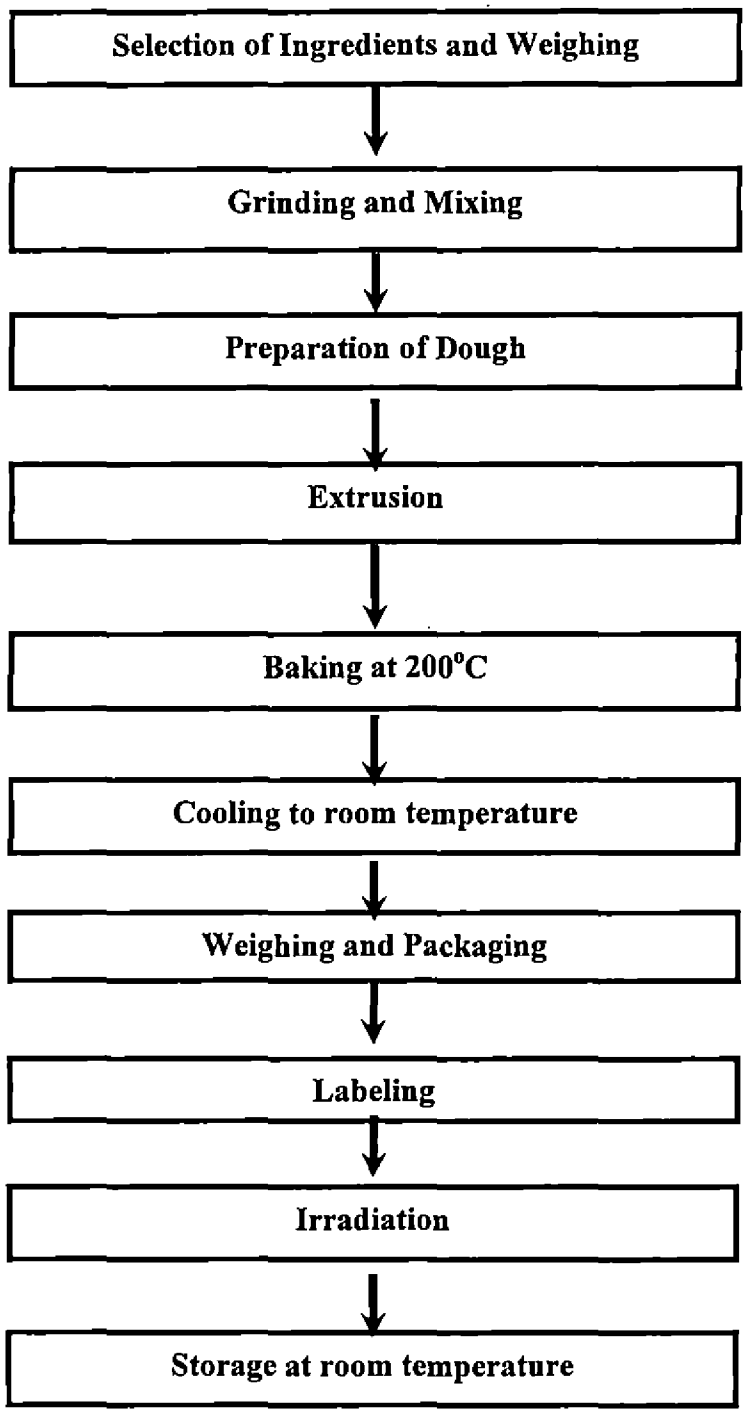
Table 1. Ingredients of the dog biscuit

INGREDIENTS	**QUANTITY** (%)
Meat cum bone meal	33.00
Plain flour (*Maida*)	24.50
Bovine Blood	24.50
Rendered fat	5.00
Egg	5.00
Bran	5.00
Black gram flour	3.00
Total	**100.00**

Table 2. Time temperature schedule for baking

% MOISTURE (approximate)	**TEMPERATURE** (^{o}C)	**TIME** (Min.)
5	200	60
10	200	35
15	200	18
25	200	7

Figure 1. Flow chart for the preparation of dog biscuit



3.2. GAMMA IRRADIATION

The packed samples of 200g were irradiated at five different dose levels of 2.0 kGy, 3.0 kGy, 4.0 kGy, 6.0 kGy and 8.0 kGy in a Gamma Chamber 5000, (BRIT-DAE, Mumbai) where the source of radiation was Cobalt 60 (^{60}Co). The packed non irradiated samples were designated as control samples.

The irradiated and non irradiated control samples were analysed for the various parameters on the day of preparation and then on 7, 15, 30, 45, 60, 75, 90, 120, 150 and 180 days of storage at ambient temperature or their spoilage which ever was earlier.

3.3. PROXIMATE ANALYSIS

The prepared dog biscuits were analyzed for their proximate compositions viz., moisture, ether extract, protein content, crude fiber and ash on different days of storage as mentioned above. One sample from each category was taken for analysis and the composition was expressed as percentage of the dog biscuit.

3.3.1. Moisture

Moisture content of the dog biscuits were analysed on the day of preparation itself to ascertain whether they attained the expected moisture level. Then the analysis was conducted on the various days of study (AOAC, 1990). A 10 g sample in a petri dish was kept in a hot air oven at $100 \pm 2^{\circ}$ C for 16 to 18 hours. The weight of the dry samples was taken after cooling in a desiccator. The difference in the weight is the moisture content of the sample and expressed as percentage of the dog biscuit.

3.3.2. Ether extract

It is the total fat content of the sample. Ether extraction was conducted as per AOAC (1990) using Socs Plus solvent extraction system (Pelican Equipments, India). Two gram moisture free sample was taken for the analysis and the fat content was expressed as percentage of the dog biscuits.

3.3.3. 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.

Crude protein % = 6.25 X % Nitrogen.

3.3.4. Crude Fibre

One gram fat free sample was transferred to a 500 ml flask. 100 ml of 0.255 N sulphuric acid was added to it and boiled for 30 minutes under water condenser as per AOAC (1990). The whole content was filtered through a muslin cloth and the residue was washed with water to free from the acid. The residue was transferred in to the flask and added 100ml 0.313 N sodium hydroxide followed by 2 drops of amyl alcohol and boiled it for 30 minutes again. The residue was filtered and transferred to a crucible. Dried in hot air oven for 2hours and weighed it. Then this was kept in muffle furnace at 600°C for 2 hours and weighed. The difference in weight was recorded as the fiber content.

3.3.5. Ash

Ash is the total mineral content of a sample. Two gram of the sample was placed in a porcelain crucible and kept in a muffle furnace at 600°C for 2 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 (AOAC, 1990)

3.3.6. Nitrogen Free Extract (NFE)

Nitrogen free extract (NFE) of the sample was calculated using the equation. (AOAC, 1990)

NFE = 100 – (moisture + ether extract + protein + crude fiber + ash).

3.3.7. Gross Energy (GE)

In case of dog foods gross energy can be found out by using all the proximate composition values. The gross energy was found out by the equation given below. (Kienzle *et al.*, 1998)

GE = (Protein X 0.24) + (Fat X 0.38) + (Carbohydrates X 0.17)

3.3.8. Calcium content

Total calcium content was estimated as per AOAC (1990) using Atomic Absorption Spectrophotometer (PERKIN ELMER 3110, US instrument division, Norwalk, USA). The reading obtained was converted to gram percentage using the formula given below.

$$\text{Calcium \%} = \frac{\text{AAS reading X dilution of the sample}}{\text{Weight of the sample X 10000}}$$

3.3.9. Phosphorus content

The phosphorus content was estimated as per AOAC (1990) using spectrophotometer in Spectronic 1001 Plus (Miton Roy Company, USA). The phosphorus content was calculated from the optical density (OD) values at 490nm using the formula given below. (AOAC, 1990)

$$\text{Phosphorus \%} = \frac{\text{OD of the sample X concentration of standard X dilution factor X 100}}{\text{OD of the standard X weight of the sample X 1000}}$$

3.4. THIOBARBITURIC ACID REACTIVE SUBSTANCE VALUES

Thiobarbituric acid reactive substances (TBARS) produced from lipid peroxidation were determined using the method of Witte *et al.,* (1970). A 20 gram powdered sample was blended with 50 ml chilled extracting solution containing 20 per cent trichloroacetic acid in 2M phosphoric acid for 1.5 to 2 minutes. The resultant solution was transferred to a 100 ml volumetric flask. Then the sample was made up to 100 ml using deionised distilled water. This solution was filtered using Whatman No.1 filter paper. Five millilitre filtrate was transferred to a screw caped glass vials followed by the same quantity of 2-thiobarbituric acid solution (0.005M in distilled water). The solution was mixed by inverting the vial and it is kept for 15 hours in darkness at room temperature. The absorbance of this solution was determined at 530 nm against blank containing 5ml distilled water and 5ml of 2-thiobarbituric acid solution (0.005M) in UV–Vis Spectrophotometer (Systronics.119, India). The absorbance was converted to TBARS values and was expressed as milligram malonaldehyde per kilogram of dog biscuit.

3.5. MICROBIOLOGICAL ANALYSIS

In order to estimate the microbial load per milligram of dog biscuit the sealed pouches were opened near a burner taking all aseptic precautions. Twenty five grams in duplicate were weighed from the irradiated and their corresponding non-irradiated control batches. These samples were aseptically homogenized for 30 seconds with sterile 225 ml of 0.01 per cent peptone water (diluent) in a stomacher (Seward stomacher® 400 circular) so as to form one in 10 dilution of the sample. Further 10 fold dilutions were prepared by transferring one millilitre of inoculum to nine millilitre of the diluents. Dilutions were made up to 10^{-8} and selected dilutions of each sample were used for the

estimation of various microbiological loads per gram of the sample. All aseptic precautions were taken during collection and processing of the samples.

Selected serial dilutions of each sample were used to estimate the load of aerobic bacteria, Coliforms, *Escherichia coli*, Faecal Streptococci, Staphylococci, Salmonella, Clostridium and Yeast and mould. The counts were expressed as $\log_{10}$ cfu/g

3.5.1. Aerobic plate count (APC)

Aerobic plate count (APC) of each sample was estimated by pour plate technique, as described by Morton (2001). From the selected ten fold dilution of each sample, one millilitre of the inoculam was transferred in to duplicate petri dishes of 100 mm diameter. To each inoculated plates about 15 to 20 ml sterile molten standard plate count agar (HiMedia, Mumbai) maintained at 45°C was poured and mixed with the inoculam, by gentle rotatory, forward and backward movements. The inoculated plates were left at room temperature and allowed to solidify, and incubated at 37°C for 24 hours in upside down position. At the end of incubation period those plates having colonies between 20 and 200 were selected and counts were taken with the help of a digital cubic colony counter (Royal, India). The number of colony forming units (cfu) per gram of the sample was calculated by taking the average of duplicate plates and multiplied by the dilution factor and converted to $\log_{10}$ cfu/g of dog biscuits.

3.5.2. Coliform and *Escherichia coli* Count

Coliform count was estimated according to the procedure described by Kornacki and Johnson (2001). From selected serial dilutions, 1 ml of the homogenized sample was inoculated in to the sterile petri plates to which 15 to 20 ml of molten Eosin Methyline Blue agar (HiMedia, Mumbai) was poured, mixed well and allowed to solidify. The plates were incubated upside down at 37°C for 24 hours. At the end of incubation period, purplish black colonies

with diameter of at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms.

E. coli were also counted from the same plate. The colonies which are showing greenish black metallic sheen on deflected light were counted as *E. coli* and the counts were expressed as $\log_{10}$ cfu/g of the sample and further confirmed by IMViC test.

3.5.3. Faecal Streptococcal count

One ml of the sample was transferred to sterile Petri dishes of uniform size. Fifteen to twenty millilitre of Karl Friedrich streptococcal agar (HiMedia, Mumbai) maintained at 45°C was added, mixed well and allowed to solidify. These plates are incubated at upside down for 48 hours at 37°C. The red or pink coloured colonies with a diameter between 0.5 and 3 millimeter and surrounded by a narrow whitish zone were counted with the help of digital colony counter and converted the counts as $\log_{10}$ cfu /g. of the sample. (Hartman *et al.*, 2001)

3.5.4. Staphylococcal count

To one ml of the sample from the selected dilution in Petri dish, added 15 to 20 ml of Baird Parker's Agar (HiMedia, Mumbai) at 45°C, mixed it well and allowed to solidify. The plates were incubated at 37°C for 48 hours. The colonies with circular, smooth, convex, 2-3 mm in diameter on uncrowded plates, gray black to jet black, frequently with light coloured margin were counted with the help of a colony counter and the counts were converted to $\log_{10}$ cfu/ g. of the sample. (Lancette and Bennett, 2001)

3.5.5. Salmonella count

Twenty five gram of aseptically weighed sample was blended in 225ml of lactose broth and pre enriched at 37°C for 24 hours. After pre enrichment 10 ml of this culture was transferred to 100 ml of Tetrathionate Brilliant Green (TBG) broth and 100ml of Rappaport Vassiliadis (RV) broth (HiMedia,

Mumbai). This enrichment broth is incubated for 24 hours at 43°C. At the end of incubation a loop full of the culture from each enrichment broth was inoculated on the duplicate plate of Brilliant Green Agar (BGA) and Salmonella Shigella (SS) agar (HiMedia, Mumbai) the plates were incubated at 37°C for 24 hours. At the end of incubation colourless pinkish white opaque to translucent colonies with a diameter of about 1-2 mm, surrounded with a pink or red hue on BGA plates and colourless colonies with black center on SS agar were counted as salmonella organisms. (Andrews *et al.*, 2001)

3.5.6. Clostridium count

To one millilitre of the sample added 12 to 15 ml of Sulfite Polymyxin Sulfadiazine agar (HiMedia, Mumbai) having a temperature of 45°C in sterile long tubes. Above the agar liquid paraffin is filled up to a height of 2 inches for making the media anaerobic. These tubes are incubated for 24 hours at 45° C. All the black coloured colonies were counted and converted the counts to $\log_{10}$ cfu / g. of the sample. (Labbe *et al.*, 2001)

3.5.7. 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 inoculam was transferred on to duplicate plates. To each plate 15 to 20 ml of molten media at 45°C was added mixed well and allowed to solidify. The plates are incubated at 25- 27°C for 3 days. After incubation colonies were counted with the help of a colony counter and mean count was multiplied with the dilution factor and expressed as $\log_{10}$ cfu/g

3.6. PALATABILITY ASSESSMENT

Palatability or acceptability is the measure of food preferences and depends on taste, texture and odour. In this study, the palatability or acceptability assessment was conducted in 18 dogs selected at random from the locality in which 9 were heavy breeds and 9 were toy / small breeds. Biscuits of all moisture levels about 15 days after preparation and irradiation were given to the dogs. Biscuits of four different moisture levels (5, 10, 15 and 25 per cent) were given separately as four stages of study. Duration of each stage was about 3 days and 2 days gap were given in between two stages. The biscuits were fed to the dogs at its normal feeding time before the routine feed was given. The reactions of the dogs towards the biscuits were observed. Observations were made giving stress towards the approach to food, interest to eat and nature of eating. The observations taken at the 3rd day of each stage were recorded in the score card (appended). The opinion of dog owners about the biscuits with respect to odour, colour and appearance were also recorded in the same score card. The data obtained were tabulated and expressed in percentage for different moisture level dog biscuits.

3.7. STATISTICAL ANALYSIS

The data obtained with respect to proximate composition, TBARS values and microbiological load were analysed statistically using completely randomized design (CRD) or one way Analysis of variance. (Snedecor and Cochran, 1994)

SCORE CARD FOR DOG BISCUIT

1. Name & Address of the owner:

2. No. of dogs owned:

3. Purpose of keeping dogs:

4. Details of the dog

   Breed:

   Sex:

   Age:

5. Score card for the owner [put a tick (√) mark on appropriate column]:

No	Parameters	***High***	***Medium***	***Low***
1.	***Odour***	Highly pleasant	Pleasant	Bad
2	**Colour & appearance**	Highly appealing	Appealing	Not appealing

6. Score card for dogs [put a tick (√) mark on appropriate column by the owner]:

No	Parameters	***High***	***Medium***	***Low***
1	**Approach to food**	Highly approaching	Approaching	Rejecting
2	***Interest to eat***	Highly interested	Eating	Not eating
3	***Nature of eating***	Gulping	Chewing	Rejecting

7. General comments by the owner:

Signature of the owner

RESULTS

Dog biscuits were prepared incorporating the various ingredients as mentioned in the Table 1 and backed at 200°C in order to attain moisture levels of 5, 10, 15 and 25 per cent. The 5 per cent moisture level samples were designated as control samples for palatability/acceptability studies after irradiation since many of the branded pet food items available in the market contain only 5 per cent moisture. Samples were separately packed in HDPE packets at a rate of 200 g each. The samples containing 10, 15 and 25 per cent moisture levels were subjected to irradiation using Gamma Chamber 5000 and irradiated at various doses of zero (R0), 2.0 kGy (R2), 3.0 kGy (R3), 4.0 kGy (R4), 6.0 kGy (R6) and 8.0 kGy (R8). After irradiation the samples were kept at ambient temperature for further analysis till visual or organoleptic spoilage were shown by the product. The analysis were conducted on the day of preparation and then on 7, 15, 30, 45, 60, 75, 90, 120, 150, 180 days of storage or till spoilage occurred whichever was earlier.

It was observed that R0 samples of 10 per cent moisture level were spoiled between 30 to 45 days of storage, the same non irradiated samples of 15 percent moisture level were spoiled between 15 to 30 days of storage in case of 25 per cent moisture level samples it was between 3 to 4 days after preparation (Table 3). The dog biscuit of 10 per cent moisture level irradiated at 4.0 kGy was the only sample which was not spoiled even after 180 days of storage study.

4.1. PROXIMATE COMPOSITION

4.1.1 Moisture Content

The moisture percentage of the three moisture level (10, 15 and 25 per cent) biscuits on the day of preparation and during the storage period is shown in the Table 4, Table 5 and Table 6 respectively. It was observed that neither the dose of irradiation nor the days of storage had any significant effect on the moisture content of the dog biscuits. The maximum percentage of moisture in 10 per cent moisture level sample on the day of preparation was 9.7 ± 0.16 and on 180th day the same sample showed 9.67 ± 0.14 (4.0 kGy) gram percentage. In case of 15 percent moisture sample the analytical studies showed the maximum per cent of moisture as 14.59 ± 0.18 and 14.57 ± 0.26 in R4 sample on 150 days of storage. The moisture percentage of 25 per cent moisture level sample had revealed the maximum of 24.52 ± 0.23 on the day of preparation and 24.53 ± 0.18 on 120th day for R8 samples.

4.1.2. Ether Extract

The fat content of 10 per cent moisture level biscuits are shown in Table 7. It was observed the fat per cent varied from 11.3 ± 0.28 to 11.23 ± 0.19. The values were non significant among treatment groups. It was also observed that there was no significant difference in fat content due to the increase in days of storage.

The fat content of 15 percent moisture containing biscuit sample were shown in Table 8. It was observed that the fat percentage varied from10.78 ± 0.11 to 10.73 ± 0.15. The values were not affected significantly by either due to the dose of irradiation or due to the days of storage.

Twenty five per cent moisture level biscuits had recorded a fat percentage between 9.48 ± 0.11 and 9.41 ± 0.052 during storage (Table 9). The values were

not significantly affected either due to irradiation or due to days of storage even though the control and irradiated samples containing 25 per cent moisture level were spoiled in the course of storage.

4.1.3. Protein

The Protein content of the 10 percent moisture level samples is shown in Table10. It varied from 28.21 ± 0.26 to 28.35 ± 0.23. It was observed that the percentage of protein was not affected significantly due to various doses of radiation so also with the days of storage. Even at 180th day of storage in R4 sample had a protein content of 28.2 ± 0.02 gram percentage.

A slight reduction in protein content was noticed in dog biscuits containing 15 percent moisture (Table 11). The maximum protein level was noticed in R2 samples on the day of preparation 27.31 ± 0.3 gram percentage. Even after 150 days of storage a non significant value was recorded in R4 samples. Neither the dose of irradiation nor the days of storage had any effect on protein percentage.

It was observed still a lower content of protein in dog biscuit containing 25 per cent moisture (Table 12). The values for protein were around 24 per cent. It was also observed like that of moisture, fat and protein content of other samples, the dose of irradiation and days of storage has not influenced the protein content of the biscuits.

4.1.4. Crude Fibre

The results of crude fibre analyzes are given on Table13, Table 14, Table15 for different moisture level. The highest value obtained for crude fibre for 10 per cent moisture value was 7.26 ± 0.19 on 30th day of storage for R2 samples, for 15 per cent moisture level dog biscuit it was 6.86 ± 0.21 on the 7th day of storage in R4 samples and 6.073 ± 0.11 on 120th day of storage for R6 samples in 25 per cent

moisture level dog biscuits. The lowest values obtained for 10 per cent moisture level dog biscuits was 7.2 ± 0.17 on the day of preparation for R3 samples, for 15 per cent moisture level dog biscuit it was 6.77 ± 0.09on the day of preparation in R2 samples and 6.04 ± 0.11 on the 7th day of storage for R6 samples in 25 per cent moisture level dog biscuits. Either the dose of radiation or the days of storage were not significant with respect to the content of crude fibre in dog biscuits. As the percentage of moisture increased there was a decreasing trend of crude fibre was also observed.

4.1.5. Ash

Results for the ash content of dog biscuits for various levels of moisture are presented in Table 16, Table 17 and Table 18. The highest value obtained for ash in 10 per cent moisture level dog biscuit was 17.09 ± 0.40 on the day of preparation for R3 samples, for 15 per cent moisture level dog biscuit shown a value of 16.17 ± 0.22 on the 7th day of storage in R0 samples and 14.21 ± 0.26 on the day of preparation for R0 samples. The lowest value obtained for 10 per cent moisture level dog biscuits was 17.04 ± 0.25 on the 7th day of storage for R4 samples, for 15 per cent moisture level dog biscuit the ash content was 16.11±0.15 on the day of preparation for R2 samples and 14.12±0.2 on the 7th day of storage for R8 samples was obtained for 25 per cent moisture level dog biscuits. The values obtained were not significant with respect to the different dose levels of irradiation. There was also no significant difference observed in ash content with respect to the storage days.

4.1.6. Nitrogen Free Extract

The NFE content of 10 per cent moisture level samples were shown in Table 19, for 15 percent moisture sample in Table20 and 25 per cent moisture level

samples are presented in Table 21. The highest value obtained for ash for 10 per cent moisture level dog biscuit was 26.58 ± 0.25 on the day of preparation for R0 samples, for 15 per cent moisture level dog biscuit it was 24.53 ± 0.68 on the 15th day of storage for R2 samples and 21.8 ± 0.21 on 7th day for R8 samples for 25 per cent moisture level biscuits. The lowest value obtained for 10 per cent moisture level dog biscuit was26.37 ± 0.10 on the day of preparation for R3 samples, for 15 per cent moisture level dog biscuit it was 24.37 ± 0.42 on 150th day of storage for R4 samples and 21.57±0.15 on 90th day for R8 samples for 25 per cent moisture dog biscuit. Similar to that of other proximate parameters studied these values were also not significant during the storage period as well as due to treatment.

4.1.7. Gross Energy

The GE content of the samples was arrived from the equation given below.

GE = (Protein X 0.24) + (Fat X 0.38) + (Carbohydrates X 0.17)

The data obtained are presented in Table22, Table 23 and Table 24. The highest values obtained for GE in10 per cent moisture level dog biscuit was 16.81 ± 0.07 on the day of preparation for R2 samples, for 15 per cent moisture level dog biscuit it was 15.96 ± 0.06 on the day of preparation for R2 samples and 9.48 ± 0.11 on 120th day of storage for R6 samples for 25 per cent moisture dog biscuits. The lowest values obtained for 10 per cent moisture level dog biscuits was 16.79 ± 0.06 on the 180th day of storage for R4 samples, for 15 per cent moisture level dog biscuit it was 15.94 ± 0.06 on 150th day of storage for R4 samples and 14.08 ± 0.03on the day of preparation for R8 samples was obtained for 25 per cent moisture level dog biscuits. It was observed non significant values on the storage as well as in treatment in all the three dog biscuits. As percentage of moisture

increased the GE available with in the biscuits reduced due to the high percentage of water content in the biscuit.

4.1.7. Calcium and Phosphorus Content

The calcium and phosphorus content of the dog biscuits in gram percentage obtained on the day of preparation has shown in the Table 25. The values were non significant due to various dose of irradiation on the day of preparation. Similar to other parameters as moisture percentage increased there was a similar reduction in calcium and phosphorus content.

4.2. THIOBARBITURIC ACID REACTIVE SUBSTANCE VALUES

The TBARS values for the samples at 10 per cent moisture level are shown in Table 26. The non irradiated control sample recorded a value of 0.32 ± 0.02 on the day of preparation. It was observed a significant ($P<0.05$) increase in TBARS values due to various doses of irradiation on the same day. During the days of storage it was observed an increasing trend in malonaldehyde per kilogram of the sample (Figure 2). By 180 days of storage in R4 samples the TBARS values reached from 0.5 ± 0.02 to 2.54 ± 0.02, at the same time in R6 and R8 samples the same values were reached by 90 days of storage.

The data obtained for TBARS values in 15 per cent moisture level samples are given in Table 27. These samples when subjected to irradiation there noticed an increasing trend with respect to TBARS values. Similarly the days of storage also had a significant ($P<0.05$) effect on TBARS values (Figure3).

The values of 25 percent moisture samples are shown in Table 28. The effect of storage on TBARS values are also shown in Figure 4. It was observed a significant ($P<0.05$) increase in TBARS due to dose of irradiation and days of

storage. The initial value of 0.65 ± 0.16 in R8 sample was increased to 3.69 ± 0.03 by 120 days of storage.

4.3. MICROBIOLOGICAL LOAD

4.3.1. Aerobic plate count

The mean and standard error of APC for 10 per cent moisture level dog biscuits expressed in $\log_{10}$ cfu/g are shown in Table 29, the similar values with respect to 15 per cent moisture samples are shown in Table 30 and the APC of 25 per cent moisture samples are shown in Table 31. It was observed a 100 per cent reduction of aerobic organisms in 10 per cent moisture level samples due to irradiation at 6.0 kGy and 8.0 kGy, where as only 8.0 kGy irradiation has destroyed the organisms completely in 15 per cent moisture level dog biscuits. It was also observed there was a significant ($P<0.05$) reduction due to irradiation at 2.0 kGy, 3.0 kGy and 4.0 kGy compared to the non irradiated control samples on the day of preparation till the day of spoilage. It was observed a significant ($P<0.05$) increase in aerobic plate count during the storage period in all samples under investigation.

In dog biscuits containing 15 per cent moisture only the samples irradiated at 8.0 kGy has destroyed total aerobic organisms. It was observed a significant ($P<0.05$) reduction due to different doses of irradiation and the trend continued till spoilage. It was noted that there was an increasing trend in the microbiological strength during the days of storage.

As evidenced by a shorter baking period, the initial count of microorganisms present in the non irradiated control samples were significantly higher since none of the treatment had made the 25 per cent moisture level sample free from aerobic micro organisms. It was observed that there was a significant ($P<0.05$) reduction in microbial load due to irradiation. An increasing log count

was observed during the storage period up to 120 days of storage for samples treated at 6.0 and 8.0 kGy.

The growth pattern of microorganisms during the storage period in different moisture level dog biscuits has shown in Figure 5, Figure 6 and Figure 7.

4.3.2. Coliform and *E. coli* count

The coliform count of the samples analysed is shown in the Table 32. It was observed that no samples at different moisture levels and treatment groups revealed *E. coli* count.

The control samples on the day of preparation recorded 4.44 ± 0.21 $\log_{10}$ cfu/g. Due to irradiation at 2.0 and 3.0 kGy the count has significantly reduced. The treatment by 4.0, 6.0 and 8.0 kGy of irradiation has made coliforms non detectable in 10, 15 and 25 per cent moisture level dog biscuits. Similarly by making the dog biscuits to 10 and 15 percent moisture level no coliforms or *E. coli* survived even in non irradiated samples. It was observed that, as the storage period of R2 and R3 samples enhanced, there was a significant ($P<0.05$) increase in coliform count in both these samples.

4.3.3. Staphylococcal count

The mean and standard error of the recorded Staphylococcal count in the dog biscuit are shown in the Table 33. The dog biscuit containing 10 and 15 per cent moisture did not reveal any staphylococcus organisms. The 25 per cent moisture level non irradiated samples revealed a log count of 4.58 ± 0.16 on the day of preparation. Due to irradiation even at this moisture level has brought the staphylococcal counts to non detectable level employing 4.0, 6.0 and 8.0 kGy. Treatment by 2.0 and 3.0 kGy significantly ($P<0.05$) reduced the staphylococcal

count on the day of preparation. By keeping the samples to 7th day it was also showed a significant ($P<0.05$) increase due to storage.

4.3.4. Faecal Streptococcal, Salmonella and Clostridium count

It was observed that none of the dog biscuits prepared in the present study at different levels of moisture, both control and irradiated, revealed Faecal Streptococcal, Salmonella and Clostridium count.

4.3.5. Yeast and mould count

The yeast and mould count of the 10 per cent moisture level biscuits are shown in Table 34. The control sample, on the day of preparation, had the maximum count of 2.48 ± 0.21. The count was significantly reduced by irradiating the same at 2.0 and 3.0 kGy. Irradiation dose of 4.0, 6.0 and 8.0 kGy has completely destroyed yeast and mould count in bog biscuits. The Figure 8 shows the trend of growth of yeast and mould in storage period. It was noticed a significant ($P<0.05$) upward trend compared to the day of preparation.

Similarly in 15 per cent moisture level samples irradiation at 4.0, 6.0 and 8.0 kGy totally destroyed the yeast and mould growth. Even 2.0 and 3.0 kGy irradiation has significantly ($P<0.05$) reduced the yeast and mould count (Table 35). As days of storage increased there was an upward trend with respect to yeast and mould count (Figure 9).

The yeast and mould count of 25 per cent moisture level sample are shown in Table 36. It was observed a total destruction of yeast and mould due to irradiation by 6.0 and 8.0 kGy. It was also observed a significant ($P<0.05$) reduction by 2.0, 3.0 and 4.0 kGy compared to non irradiated control samples. A similar trend as that of 10 and 15 per cent samples noticed during the days of

storage (Figure 10).It was found a 5 log reduction in 4.0 kGy irradiation when compared with the control samples.

4.4. PALATABILITY

Dog biscuits containing 5, 10, 15 and 25 percent moisture level were prepared, packed in 200g packets and subjected to 4.0 kGy irradiation considering the maximum keeping quality. The palatability/acceptability were studied with the help of a score card comprising of two parts. The first part contains the remarks of the owner of animal and a second part which has to be noticed by the owner while feeding the dogs. All the attributes were classified as high, medium and low categories. The 18 dogs subjected to study were equally selected 9 numbers each in heavy breeds and small breeds. The data obtained are shown in Table 37. As the percentage of moisture increased, dogs were shown high level of approach, interest to eat and nature of eating towards such food when compared to conventional 5 percent or 10 percent level dog foods. The odour, colour and appearance marked by the owner were predominantly in medium aspects. The nature of eating in case of 5 per cent moisture level was 100 percentage medium, 95 percentage medium in 10 percent moisture where as it was 33 percent high in case of 25 percent moisture level biscuits and 83 per cent of dogs reported high interest in eating the food (Figure 11). A very good approach was recorded by 67 per cent of dog owners for 25 percent level dog biscuits.

Table 3. Shelf life of dog biscuits

Samples (in percentage)	SAMPLES	DAYS OF SPOILAGE
10	R0	30 to 45 days
	R2	45 to 60
	R3	75 to 90
	R4	Beyond 180
	R6	150 to180
	R8	150 to 180
15	R0	15 to 30
	R2	30 to 45
	R3	30 to 45
	R4	150 to 180
	R6	120 to 150
	R8	120 to 150
25	R0	3 to 4
	R2	7 to 15
	R3	7 to 15
	R4	15 to 30
	R6	120 to 150
	R8	120 to 150

R with numerical indicates the irradiation dose in kGy

Table 4. Moisture Content of dog biscuit at 10 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	9.63±0.15	9.66±0.16	9.68±0.14	9.68±0.20	S	S	S	S	S	S	S
R2	9.62±0.17	9.65±0.21	9.66±0.147	9.64±0.17	9.63±0.23	S	S	S	S	S	S
R3	9.7±0.16	9.65±0.21	9.66±0.204	9.65±0.19	9.63±0.22	9.65±0.19	9.65±0.16	S	S	S	S
R4	9.6±0.16	9.63±0.17	9.69±0.22	9.66±0.24	9.66±0.13	9.66±0.13	9.66±0.19	9.68±0.18	9.68±0.19	9.66±0.24	9.67±0.14
R6	9.65±0.16	9.64±0.14	9.64±0.16	9.65±0.18	9.65±0.13	9.65±0.16	9.66±0.16	9.66±0.14	9.65±0.17	9.6±0.19	S
R8	9.63±0.16	9.64±0.19	9.64±0.16	9.64±0.20	9.66±0.18	9.65±0.13	9.65±0.20	9.65±0.24	9.66±0.22	9.65±0.12	S

Table 5. Moisture Content of dog biscuit at 15 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	14.58±0.19	14.54±0.22	14.57±0.21	S	S	S	S	S	S	S
R2	14.55±0.24	14.52±0.27	14.51±0.25	14.56±0.27	S	S	S	S	S	S
R3	14.59±0.18	14.54±0.24	14.54±0.20	14.57±0.17	S	S	S	S	S	S
R4	14.55±0.31	14.59±0.22	14.58±0.23	14.56±0.23	14.54±0.21	14.52±0.22	14.55±0.32	14.56±0.24	14.56±0.2	14.57±0.26
R6	14.55±0.24	14.55±0.21	14.55±0.17	14.55±0.27	14.55±0.27	14.55±0.18	14.55±0.17	14.55±0.17	14.55±0.21	S
R8	14.56±0.20	14.56±0.15	14.55±0.17	14.55±0.18	14.54±0.25	14.54±0.18	14.55±0.22	14.55±0.15	14.56±0.19	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 6 Moisture Content of dog biscuit at 25 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	24.46±0.24	S	S	S	S	S	S	S	S
R2	24.5±0.21	24.53±0.34	S	S	S	S	S	S	S
R3	24.51±0.22	24.52±0.16	S	S	S	S	S	S	S
R4	24.5±0.18	24.51±0.22	24.56±0.21	S	S	S	S	S	S
R6	24.49±0.28	24.51±0.19	24.54±0.30	24.53±0.16	24.52±0.22	24.57±0.25	24.56±0.23	24.53±0.14	24.46±0.17
R8	24.52±0.23	24.51±0.18	24.51±0.17	24.51±0.08	24.53±0.18	24.55±0.23	24.53±0.16	24.54±0.30	24.53±0.18

Table 7. Fat content of dog biscuit at 10 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	11.26±0.21	11.23±0.14	11.25±0.17	11.25±0.27	S	S	S	S	S	S	S
R2	11.30±0.16	11.24±0.18	11.24±0.21	11.25±0.24	11.26±0.23	S	S	S	S	S	S
R3	11.3±0.28	11.23±0.27	11.26±0.28	11.24±0.28	11.24±0.20	11.23±0.16	11.24±0.24	S	S	S	S
R4	11.25±0.19	11.24±0.22	11.26±0.23	11.24±0.21	11.24±0.22	11.24±0.22	11.25±0.28	11.24±0.21	11.25±0.29	11.24±0.27	11.24±0.21
R6	11.23±0.19	11.26±0.21	11.25±0.16	11.25±0.18	11.25±0.19	11.25±0.16	11.25±0.18	11.24±0.20	11.24±0.16	11.24±0.21	S
R8	11.26±0.17	11.24±0.18	11.25±0.23	11.24±0.22	11.26±0.21	11.25±0.24	11.24±0.30	11.24±0.22	11.24±0.27	11.24±0.25	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 8. Fat Content of dog biscuit at 15 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	10.75±0.17	10.76±0.16	10.76±0.14	S	S	S	S	S	S	S
R2	10.76±0.16	10.74±0.18	10.75±0.17	10.73±0.15	S	S	S	S	S	S
R3	10.76±0.22	10.77±0.15	10.76±0.14	10.76±0.16	S	S	S	S	S	S
R4	10.77±0.16	10.78±0.11	10.76±0.13	10.77±0.14	10.75±0.17	10.74±0.11	10.77±0.16	10.74±0.15	10.77±0.17	10.75±0.12
R6	10.76±0.08	10.75±0.10	10.75±0.11	10.76±0.12	10.75±0.13	10.75±0.16	10.74±0.15	10.74±0.14	10.75±0.12	S
R8	10.75±0.12	10.76±0.13	10.75±0.14	10.76±0.14	10.76±0.11	10.75±0.13	10.76±0.09	10.75±0.17	10.75±0.14	S

Table 9. Fat Content of dog biscuit at 25 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	9.45±0.092	S	S	S	S	S	S	S	S
R2	9.41±0.052	9.41±0.17	S	S	S	S	S	S	S
R3	9.47±0.12	9.42±0.05	S	S	S	S	S	S	S
R4	9.48±0.11	9.41±0.10	9.44±0.19	S	S	S	S	S	S
R6	9.47±0.12	9.44±0.12	9.48±0.15	9.46±0.06	9.46±0.13	9.45±0.17	9.43±0.20	9.46±0.09	9.45±0.05
R8	9.47±0.11	9.44±0.06	9.45±0.11	9.45±0.12	9.46±0.11	9.45±0.15	9.46±0.08	9.46±0.11	9.45±0.13

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 10. Protein Content of dog biscuit at 10 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	28.22±0.22	28.21±0.26	28.25±0.25	28.25±0.19	S	S	S	S	S	S	S
R2	28.24±0.34	28.25±0.22	28.24±0.23	28.24±0.17	28.26±0.27	S	S	S	S	S	S
R3	28.31±0.22	28.24±0.19	28.25±0.20	28.25±0.20	28.26±0.20	28.25±0.22	28.23±0.23	S	S	S	S
R4	28.35±0.23	28.25±0.21	28.26±0.27	28.26±0.24	28.26±0.22	28.25±0.26	28.24±0.18	28.25±0.21	28.21±0.24	28.27±0.16	28.22±0.23
R6	28.26±0.21	28.24±0.17	28.25±0.20	28.25±0.19	28.25±0.17	28.25±0.26	28.24±0.18	28.24±0.19	28.24±0.22	28.26±0.19	S
R8	28.26±0.25	28.24±0.17	28.25±0.23	28.26±0.26	28.25±0.22	28.25±0.25	28.24±0.20	28.24±0.24	28.24±0.18	28.24±0.24	S

Table 11. Protein Content of dog biscuit at 15 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	27.25±0.24	27.24±0.23	27.22±0.19	S	S	S	S	S	S	S
R2	27.31±0.30	27.23±0.24	27.22±0.24	27.24±0.28	S	S	S	S	S	S
R3	27.26±0.27	27.25±0.26	27.22±0.27	27.23±0.23	S	S	S	S	S	S
R4	27.25±0.32	27.24±0.27	27.23±0.27	27.25±0.24	27.24±0.22	27.24±0.34	27.23±0.33	27.24±0.28	27.25±0.36	27.27±0.34
R6	27.26±0.31	27.25±0.25	27.24±0.24	27.25±0.33	27.25±0.25	27.24±0.17	27.24±0.20	27.24±0.19	27.24±0.32	S
R8	27.25±0.18	27.24±0.29	27.25±0.29	27.26±0.17	27.25±0.18	27.25±0.28	27.23±0.29	27.25±0.29	27.24±0.24	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 12. Protein Content of dog biscuit at 25 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	24.07±0.15	S	S	S	S	S	S	S	S
R2	24.09±0.10	24.09±0.12	S	S	S	S	S	S	S
R3	24.1±0.20	24.09±0.11	S	S	S	S	S	S	S
R4	24.02±0.18	24.09±0.11	24.09±0.14	S	S	S	S	S	S
R6	24.03±0.10	24.07±0.14	24.07±0.14	24.07±0.03	24.06±0.13	24.06±0.22	24.07±0.17	24.17±0.08	24.18±0.10
R8	24.05±0.19	24.06±0.09	24.06±0.12	24.07±0.11	24.06±0.23	24.06±0.28	24.07±0.06	24.17±0.11	24.18±0.15

Table 13. Fiber Content of dog biscuit at 10 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	7.23±0.13	7.23±0.16	7.23±0.19	7.23±0.24	S	S	S	S	S	S	S
R2	7.21±0.17	7.23±0.14	7.26±0.19	7.23±0.22	7.22±0.19	S	S	S	S	S	S
R3	7.2±0.17	7.24±0.21	7.26±0.20	7.26±0.16	7.23±0.12	7.23±0.16	7.24±0.19	S	S	S	S
R4	7.22±0.18	7.23±0.25	7.26±0.24	7.26±0.23	7.23±0.19	7.25±0.26	7.25±0.22	7.25±0.23	7.24±0.18	7.25±0.22	7.22±0.18
R6	7.22±0.15	7.24±0.21	7.25±0.20	7.26±0.19	7.24±0.19	7.24±0.13	7.24±0.21	7.25±0.15	7.24±0.17	7.25±0.17	S
R8	7.24±0.20	7.23±0.16	7.25±0.13	7.25±0.19	7.25±0.28	7.24±0.09	7.24±0.15	7.24±0.24	7.24±0.21	7.24±0.19	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 14. Fiber Content of dog biscuit at 15 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	6.8±0.10	6.82±0.10	6.82±0.09	S	S	S	S	S	S	S
R2	6.77±0.09	6.8±0.14	6.8±0.14	6.80±0.13	S	S	S	S	S	S
R3	6.81±0.16	6.8±0.15	6.83±0.10	6.81±0.14	S	S	S	S	S	S
R4	6.84±0.23	6.86±0.21	6.85±0.18	6.84±0.22	6.84±0.12	6.83±0.09	6.85±0.21	6.82±0.17	6.84±0.13	6.85±0.16
R6	6.81±0.13	6.82±0.11	6.82±0.081	6.82±0.12	6.83±0.14	6.83±0.12	6.84±0.06	6.81±0.11	6.84±0.11	S
R8	6.82±0.14	6.81±0.10	6.81±0.081	6.82±0.12	6.83±0.13	6.83±0.14	6.83±0.16	6.81±0.11	6.83±0.10	S

Table 15. Fiber Content of dog biscuit at 25 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	6.07±0.098	S	S	S	S	S	S	S	S
R2	6.06±0.038	6.06±0.06	S	S	S	S	S	S	S
R3	6.05±0.087	6.05±0.08	S	S	S	S	S	S	S
R4	6.05±0.083	6.05±0.06	6.05±0.14	S	S	S	S	S	S
R6	6.06±0.058	6.04±0.11	6.04±0.13	6.06±0.09	6.06±0.20	6.06±0.21	6.06±0.12	6.06±0.07	6.073±0.11
R8	6.06±0.075	6.05±0.06	6.06±0.08	6.05±0.08	6.05±0.14	6.06±0.25	6.07±0.07	6.06±0.13	6.07±0.08

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

TABLE 16. Ash Content of dog biscuit at 10 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	14.21±0.26	S	S	S	S	S	S	S	S
R2	14.14±0.32	14.14±0.26	S	S	S	S	S	S	S
R3	14.2±0.22	14.14±0.20	S	S	S	S	S	S	S
R4	14.19±0.17	14.14±0.16	14.13±0.24	S	S	S	S	S	S
R6	14.19±0.22	14.18±0.10	14.16±0.13	14.17±0.05	14.17±0.15	14.16±0.20	14.17±0.15	14.17±0.19	14.18±0.24
R8	14.19±0.25	14.12±0.20	14.16±0.05	14.18±0.12	14.17±0.13	14.16±0.26	14.17±0.11	14.17±0.14	14.16±0.24

Table 17. Ash Content of the 15 per cent moisture level sample (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	16.12±0.20	16.17±0.22	16.15±0.19	S	S	S	S	S	S	S
R2	16.11±0.15	16.16±0.18	16.17±0.19	16.14±0.14	S	S	S	S	S	S
R3	16.11±0.28	16.13±0.22	16.15±0.20	16.15±0.29	S	S	S	S	S	S
R4	16.11±0.29	16.13±0.24	16.13±0.26	16.15±0.29	16.17±0.27	16.16±0.35	16.14±0.24	16.16±0.20	16.14±0.21	16.16±0.23
R6	16.11±0.25	16.11±0.20	16.12±0.18	16.13±0.25	16.14±0.27	16.14±0.11	16.13±0.15	16.14±0.18	16.14±0.17	S
R8	16.11±0.20	16.11±0.18	16.11±0.16	16.13±0.15	16.14±0.16	16.14±0.24	16.13±0.17	16.14±0.24	16.13±0.19	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 18. Ash Content of dog biscuit at 25 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	17.05±0.13	17.05±0.21	17.04±0.26	17.05±0.25	S	S	S	S	S	S	S
R2	17.06±0.23	17.04±0.26	17.05±0.26	17.07±0.21	17.06±0.24	S	S	S	S	S	S
R3	17.09±0.40	17.04±0.28	17.04±0.22	17.05±0.26	17.05±0.26	17.06±0.09	17.06±0.26	S	S	S	S
R4	17.08±0.27	17.04±0.25	17.04±0.24	17.05±0.25	17.05±0.27	17.06±0.26	17.06±0.24	17.05±0.21	17.06±0.28	17.06±0.26	17.06±0.25
R6	17.06±0.13	17.05±0.22	17.05±0.21	17.06±0.23	17.04±0.20	17.05±0.21	17.05±0.24	17.05±0.19	17.05±0.21	17.05±0.19	S
R8	17.05±0.17	17.05±0.23	17.05±0.20	17.05±0.24	17.06±0.25	17.05±0.07	17.05±0.24	17.05±0.23	17.05±0.20	17.05±0.28	S

Table 19. NFE Content of dog biscuit at 10 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	26.58±0.25	26.59±0.27	26.51±0.15	26.51±0.57	S	S	S	S	S	S	S
R2	26.55±0.39	26.57±0.54	26.51±0.42	26.55±0.45	26.55±0.66	S	S	S	S	S	S
R3	26.37±0.10	26.57±0.37	26.51±0.48	26.52±0.57	26.56±0.53	26.56±0.25	26.56±0.35	S	S	S	S
R4	26.47±0.37	26.57±0.33	26.46±0.66	26.51±0.64	26.53±0.39	26.52±0.29	26.51±0.57	26.50±0.45	26.54±0.39	26.49±0.31	26.56±0.39
R6	26.55±0.49	26.56±0.37	26.54±0.39	26.51±0.26	26.54±0.38	26.54±0.47	26.54±0.50	26.53±0.22	26.54±0.64	26.52±0.27	S
R8	26.55±0.12	26.58±0.67	26.53±0.46	26.54±0.62	26.50±0.59	26.53±0.25	26.55±0.78	26.55±0.65	26.54±0.24	26.55±0.13	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 20. NFE Content of dog biscuit at 15 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	24.49±0.38	24.44±0.45	24.46±0.39	S	S	S	S	S	S	S
R2	24.47±0.32	24.52±0.71	24.53±0.68	24.51±0.28	S	S	S	S	S	S
R3	24.43±0.25	24.47±0.36	24.47±0.32	24.44±0.41	S	S	S	S	S	S
R4	24.45±0.60	24.38±0.57	24.42±0.63	24.40±0.43	24.44±0.44	24.48±0.56	24.42±0.50	24.45±0.46	24.42±0.40	24.37±0.42
R6	24.49±0.44	24.50±0.48	24.50±0.54	24.47±0.50	24.45±0.45	24.47±0.24	24.48±0.22	24.5±0.30	24.46±0.32	S
R8	24.47±0.20	24.50±0.57	24.50±0.60	24.47±0.11	24.46±0.18	24.46±0.25	24.47±0.49	24.48±0.25	24.47±0.27	S

Table 21. NFE Content of dog biscuit at 25 per cent moisture (percentage)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	21.76±0.48	S	S	S	S	S	S	S	S
R2	21.79±0.34	21.76±0.38	S	S	S	S	S	S	S
R3	21.68±0.35	21.77±0.24	S	S	S	S	S	S	S
R4	21.75±0.33	21.78±0.33	21.71± 0.28	S	S	S	S	S	S
R6	21.75±0.59	21.75±0.27	21.68± 0.17	21.69± 0.23	21.71±0.32	21.69±0.34	21.68±0.17	21.58±0.18	21.64±0.14
R8	21.70±0.56	21.80±0.21	21.74± 0.15	21.72± 0.26	21.7 ±0.18	21.69±0.30	21.68±0.16	21.57±0.15	21.58±0.29

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 22. Gross energy of dog biscuit at 10 per cent moisture (kJ/g)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS	Day 180 NS
R0	16.8 ±0.05	16.79 ±0.07	16.79 ±0.09	16.79 ±0.07	S	S	S	S	S	S	S
R2	16.81 ±0.07	16.8 ±0.07	16.79 ±0.08	16.79 ±0.08	16.8 ±0.04	S	S	S	S	S	S
R3	16.8 ±0.12	16.79 ±0.08	16.8 ±0.06	16.79 ±0.07	16.8 ±0.069	16.79 ±0.06	16.79 ±0.1	S	S	S	S
R4	16.81 ±0.09	16.8 ±0.07	16.8 ±0.05	16.79 ±0.08	16.79 ±0.09	16.79 ±0.09	16.79 ±0.06	16.79 ±0.06	16.79 ±0.07	16.79 ±0.1	16.79 ±0.06
R6	16.79 ±0.05	16.8 ±0.08	16.8 ±0.06	16.79 ±0.07	16.8 ±0.05	16.8 ±0.08	16.79 ±0.07	16.79 ±0.06	16.79 ±0.06	16.8 ±0.08	S
R8	16.8 ±0.06	16.79 ±0.04	16.8 ±0.06	16.8 ±0.06	16.79 ±0.07	16.79 ±0.05	16.79 ±0.07	16.79 ±0.06	16.79 ±0.06	16.79 ±0.1	S

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 23. Gross energy of dog biscuit at 15 per cent moisture (kJ/g)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS	Day 150 NS
R0	15.95±0.06	15.94±0.06	15.94±0.05	S	S	S	S	S	S	S
R2	15.96±0.06	15.94±0.03	15.94±0.03	15.94±0.06	S	S	S	S	S	S
R3	15.95±0.06	15.95±0.05	15.94±0.06	15.94±0.07	S	S	S	S	S	S
R4	15.96±0.13	15.94±0.09	15.94±0.09	15.94±0.08	15.94±0.06	15.94±0.23	15.95±0.1	15.94±0.04	15.94±0.06	15.94±0.06
R6	15.95±0.07	15.95±0.05	15.95±0.03	15.95±0.09	15.94±0.1	15.94±0.08	15.94±0.03	15.94±0.05	15.94±0.03	S
R8	15.95±0.03	15.95±0.04	15.95±0.04	15.95±0.04	15.95±0.05	15.94±0.03	15.94±0.07	15.94±0.08	15.94±0.06	S

Table 24. Gross energy of dog biscuit at 25 per cent moisture (kJ/g)

SAMPLES	Day 0 NS	Day 7 NS	Day 15 NS	Day 30 NS	Day 45 NS	Day 60 NS	Day 75 NS	Day 90 NS	Day 120 NS
R0	14.09±0.06	S	S	S	S	S	S	S	S
R2	14.09±0.06	14.08±0.04	S	S	S	S	S	S	S
R3	14.09±0.06	14.09±0.05	S	S	S	S	S	S	S
R4	14.09±0.05	14.09±0.04	14.08±0.04	S	S	S	S	S	S
R6	14.09±0.05	14.08±0.05	14.09±0.07	14.09±0.03	14.09±0.05	14.08±0.07	14.07±0.08	14.09±0.04	14.1±0.03
R8	14.08±0.03	14.09±0.06	14.09±0.04	14.09±0.03	14.09±0.03	14.08±0.07	14.09±0.05	14.09±0.07	14.1±0.07

S – Spoiled NS- Non Significant R with numerical indicates the irradiation dose in kGy

Table 25.Calcium and phosphorus content of the dog biscuits at various radiation doses (percentage)

samples	25 per cent moisture sample		15 per cent moisture sample		10 per cent moisture sample	
	Calcium^{NS}	Phosphorus^{NS}	Calcium^{NS}	Phosphorus^{NS}	Calcium^{NS}	Phosphorus^{NS}
R0	2.74±0.049	1.48±0.050	3.5±0.045	1.92±0.033	3.3±0.054	1.65±0.04
R2	2.74±0.044	1.48±0.036	3.5±0.076	1.91±0.037	3.3±0.066	1.65±0.039
R3	2.74±0.037	1.48±0.035	3.5±0.057	1.91±0.046	3.3±0.053	1.65±0.034
R4	2.74±0.042	1.48±0.031	3.5±0.045	1.91±0.051	3.28±0.063	1.64±0.042
R6	2.74±0.050	1.48±0.030	3.5±0.055	1.91±0.047	3.28±0.06	1.64±0.022
R8	2.74±0.038	1.48±0.042	3.5±0.069	1.91±0.053	3.28±0.064	1.64±0.027

^{NS}- Non Significant

R with numerical indicates the irradiation dose in kGy

Table 26 TBARS values of dog biscuit at 10 per cent moisture (mg malonaldehyde /kg)

SAMPLES	Day 0	Day 7	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90	Day 120	Day 150	Day 180
R0	0.32 $\pm 0.02^{a}$	0.44 $\pm 0.01^{a}$	0.67 $\pm 0.015^{a}$	0.85 $\pm 0.01^{a}$	S	S	S	S	S	S	S
R2	0.48 $\pm 0.02^{b}$	0.65 $\pm 0.02^{b}$	0.95 $\pm 0.029^{b}$	1.19 $\pm 0.03^{b}$	1.52 $\pm 0.02^{ab}$	S	S	S	S	S	S
R3	0.49 $\pm 0.02^{bc}$	0.66 $\pm 0.02^{b}$	1.0 $\pm 0.027^{bc}$	1.27 $\pm 0.03^{b}$	1.49 $\pm 0.04^{a}$	1.78 $\pm 0.04^{a}$	1.88 $\pm 0.04^{a}$	S	S	S	S
R4	0.5 $\pm 0.02^{bc}$	0.68 $\pm 0.02^{b}$	1.06 $\pm 0.019^{c}$	1.29 $\pm 0.03^{b}$	1.59 $\pm 0.03^{bc}$	1.83 $\pm 0.04^{ab}$	1.97 $\pm 0.05^{ab}$	2.15 $\pm 0.03^{a}$	2.25 $\pm 0.04^{a}$	2.37 $\pm 0.04^{a}$	2.54 ± 0.02
R6	0.54 $\pm 0.02^{c}$	0.8 $\pm 0.02^{b}$	1.07 $\pm 0.019^{c}$	1.28 $\pm 0.03^{b}$	1.6 $\pm 0.02^{c}$	1.87 $\pm 0.05^{b}$	2.04 $\pm 0.035^{b}$	2.48 $\pm 0.03^{b}$	2.84 $\pm 0.05^{b}$	3.61 $\pm 0.03^{b}$	S
R8	0.54 $\pm 0.01^{c}$	0.68 $\pm 0.01^{b}$	1.06 $\pm 0.023^{c}$	1.27 $\pm 0.04^{b}$	1.60 $\pm 0.02^{c}$	1.88 $\pm 0.05^{b}$	2.04 $\pm 0.03^{b}$	2.48 $\pm 0.03^{b}$	2.83 $\pm 0.05^{b}$	3.61 $\pm 0.03^{b}$	S

S – Spoiled ^{NS}- Non Significant R with numerical indicates the irradiation dose in kGy

Observations having same superscript in the same column are not significantly different

Table 27. TBARS values of dog biscuit at 15 per cent moisture (mg malonaldehyde /kg)

SAMPLES	Day 0	Day 7	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90	Day 120	Day 150
R0	0.32 ± 0.02^{a}	0.42 ± 0.01^{a}	0.69 ± 0.02^{a}	S	S	S	S	S	S	S
R2	0.47 ± 0.01^{b}	0.72 ± 0.02^{b}	1.13 ± 0.04^{b}	1.37 ± 0.04^{a}	S	S	S	S	S	S
R3	0.49 ± 0.01^{b}	0.74 ± 0.02^{b}	1.18 ± 0.05^{b}	1.43 ± 0.03^{ab}	S	S	S	S	S	S
R4	0.51 ± 0.02^{bc}	0.74 ± 0.02^{bc}	1.19 ± 0.03^{b}	1.49 ± 0.03^{bc}	1.7 ± 0.05^{a}	1.91 ± 0.03^{a}	2.17 ± 0.04^{a}	2.32 ± 0.04^{a}	2.71 ± 0.05^{a}	3.59 ± 0.09
R6	0.56 ± 0.02^{c}	0.78 ± 0.02^{bc}	1.19 ± 0.05^{b}	1.55 ± 0.02^{c}	1.76 ± 0.02^{b}	1.93 ± 0.03^{ab}	2.54 ± 0.03^{b}	2.83 ± 0.02^{b}	3.77 ± 0.07^{b}	S
R8	0.63 ± 0.03^{c}	0.82 ± 0.04^{c}	1.24 ± 0.04^{b}	1.55 ± 0.02^{c}	1.76 ± 0.03^{b}	1.96 ± 0.04^{b}	2.54 ± 0.03^{b}	2.84 ± 0.04^{b}	3.78 ± 0.05^{b}	S

Table 28. TBARS values of dog biscuit at 25 per cent moisture (mg malonaldehyde / kg)

SAMPLES	Day 0	Day 7	Day 15	Day 30^{NS}	Day 45^{NS}	Day 60^{NS}	Day 75^{NS}	Day 90^{NS}	Day 120^{NS}
R0	0.32 ± 0.02^{a}	S	S	S	S	S	S	S	S
R2	0.48 ± 0.01^{b}	0.69 ± 0.01^{a}	S	S	S	S	S	S	S
R3	0.49 ± 0.01^{b}	0.7 ± 0.01^{ab}	S	S	S	S	S	S	S
R4	0.5 ± 0.01^{b}	0.72 ± 0.01^{bc}	1.17 ± 0.03^{a}	S	S	S	S	S	S
R6	0.58 ± 0.01^{c}	0.74 ± 0.01^{c}	1.21 ± 0.02^{ab}	1.49 ± 0.01	1.73 ± 0.03	1.96 ± 0.02	2.36 ± 0.04	2.86 ± 0.03	4 ± 0.04
R8	0.65 ± 0.02^{d}	0.8 ± 0.01^{d}	1.25 ± 0.02^{b}	1.51 ± 0.02	1.74 ± 0.02	1.99 ± 0.02	2.4 ± 0.03	2.85 ± 0.04	3.97 ± 0.04

S – Spoiled ^{NS}- Non Significant R with numerical indicates the irradiation dose in kGy
Observations having same superscript in the same column are not significantly different

Table 29. Aerobic plate count of dog biscuit at 10 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90	Day 120	Day 150	Day 180
R0	3.64±0.18^{a}	4.62±0.10^{a}	5.22±0.22^{a}	7.35±0.22^{a}	S	S	S	S	S	S	S
R2	2.66±0.13^{b}	3.36±0.18^{b}	4.61±0.18^{b}	5.24±0.26^{b}	7.23±0.26^{a}	S	S	S	S	S	S
R3	1.71±0.12^{c}	2.01±0.25^{c}	2.79±0.13^{c}	3.7±0.14^{c}	4.76±0.11^{b}	5.79±0.13^{a}	7.36±0.22^{a}	S	S	S	S
R4	1.04±0.2^{d}	1.81±0.12^{c}	1.96±0.15^{d}	2.34±0.08^{d}	2.62±0.14^{c}	2.78±0.16^{b}	2.83±0.16^{b}	3.18±0.2	3.36±0.1	3.62±0.15	3.79±0.2

Table 30. Aerobic plate count of dog biscuit at 15 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7	Day 15	Day 30	Day 45^{NS}	Day 60^{NS}	Day 75^{NS}	Day 90^{NS}	Day 120^{NS}	Day 150^{NS}
R0	4.57±0.18^{a}	5.85±0.25^{a}	7.35±0.28^{a}	S	S	S	S	S	S	S
R2	3.62±0.18^{b}	4.57±0.2^{b}	5.25±0.26^{b}	7.23±0.26^{a}	S	S	S	S	S	S
R3	2.58±0.15^{c}	3.49±0.21^{c}	5.15±0.19^{b}	6.78±0.11^{b}	S	S	S	S	S	S
R4	1.6±0.14^{d}	1.95±0.26^{d}	2.64±0.08^{c}	2.82±0.08^{c}	3.3±0.14	3.49±0.16	3.59±0.18	3.62±0.25	4.05±0.23	4.31±0.23
R6	1.06±0.22^{d}	1.81±0.12^{d}	2.28±0.17^{c}	2.57±0.12^{c}	2.85±0.06	3.22±0.09	3.44±0.12	3.64±0.11	3.79±0.09	S

Table 31. Aerobic plate count of dog biscuit at 25 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7	Day 15	Day 30^{NS}	Day 45^{NS}	Day 60^{NS}	Day 75^{NS}	Day 90^{NS}	Day 120^{NS}
R0	7.38±0.23^{a}	S	S	S	S	S	S	S	S
R2	5.48±0.16^{b}	7.56±0.2^{a}	S	S	S	S	S	S	S
R3	4.49±0.18^{c}	6.44±0.24^{b}	S	S	S	S	S	S	S
R4	3.8±0.12^{d}	4.23±0.28^{c}	6.59±0.19^{a}	S	S	S	S	S	S
R6	2.48±0.08^{e}	2.59±0.23^{d}	3.08±0.20^{b}	3.55±0.17	4.35±0.17	4.51±0.16	4.67±0.24	4.97±0.17	5.34±0.17
R8	1.69±0.08^{f}	2.4±0.18^{d}	2.66±0.20^{b}	3.13±0.18	3.56±0.16	4.02±0.23	4.69±0.11	4.79±0.13	4.86±0.20

S – Spoiled ^{NS}- Non Significant R with numerical indicates the irradiation dose in kGy

Observations having same superscript in the same column are not significantly different

Table 32 Coliform count of dog biscuit at 25 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7
R0	4.44±0.21[a]	S
R2	3.48±0.25[b]	4.36±0.2[a]
R3	1.69±0.14[c]	3.83±0.09[b]

Table 33. Staphylococcal count of dog biscuit at 25 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7
R0	4.58±0.16[a]	S
R2	2.51±0.17[b]	3.7±0.13[a]
R3	1.79±0.13[c]	2.4±0.26[b]

Table 34. Yeast and mould count of dog biscuit at 10 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7	Day 15	Day 30	Day 45	Day 60
R0	2.48±0.21[a]	3.69±0.12[a]	5.3±0.25[a]	6.13±0.25[a]	S	S
R2	1.58±0.12[b]	2.49±0.13[b]	3.56±0.24[b]	4.65±0.17[b]	6.36±0.22[a]	S
R3	1.08±0.23[c]	1.69±0.16[c]	2.54±0.11[c]	3.15±0.19[c]	3.95±0.21[b]	5.32±0.12

S – Spoiled [NS]- Non Significant

R with numerical indicates the irradiation dose in kGy

Observations having same superscript in the same column are not significantly different

Table 35. Yeast and mould count of dog biscuit at 15 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7	Day 15	Day 30
R0	3.46±0.17^{a}	5.3±0.25^{a}	6.21±0.31^{a}	S
R2	2.55±0.09^{b}	3.71±0.17^{b}	4.47±0.16^{b}	6.36±0.21^{a}
R3	1.42±0.26^{c}	2.48±0.13^{c}	3.7±0.14^{c}	5.5±0.19^{b}

Table 36. Yeast and mould count of dog biscuit at 25 per cent moisture (Log_{10} cfu/g)

SAMPLES	Day 0	Day 7	Day 15^{NS}
R0	6.59±0.16^{a}	S	S
R2	4.52±0.22^{b}	5.65±0.18^{a}	S
R3	2.89±0.12^{c}	4.34±0.23^{b}	S
R4	1.72±0.14^{d}	3.72±0.09^{c}	5.65±0.18

S – Spoiled ^{NS}- Non Significant

R with numerical indicates the irradiation dose in kGy

Observations having same superscript in the same column are not significantly different

Table 37. Palatability score for dog biscuits (percentage)

Samples (%)	Attributes	High (%)	Medium (%)	Low (%)
5	**Odour**		100	
	Colour & Appearance		39	61
	Approach	5	95	
	Interest	17	78	5
	Nature of Eating		100	
10	**Odour**	5	95	
	Colour & Appearance	5	33	62
	Approach	5	95	
	Interest	22	73	5
	Nature of Eating	5	95	
15	**Odour**		100	
	Colour & Appearance		44	56
	Approach	22	78	
	Interest	61	39	
	Nature of Eating	17	83	
25	**Odour**	5	95	
	Colour & Appearance		39	61
	Approach	67	33	
	Interest	83	17	
	Nature of Eating	33	67	

Figure2.TBARS value of 10 per cent moisture level dog biscuit

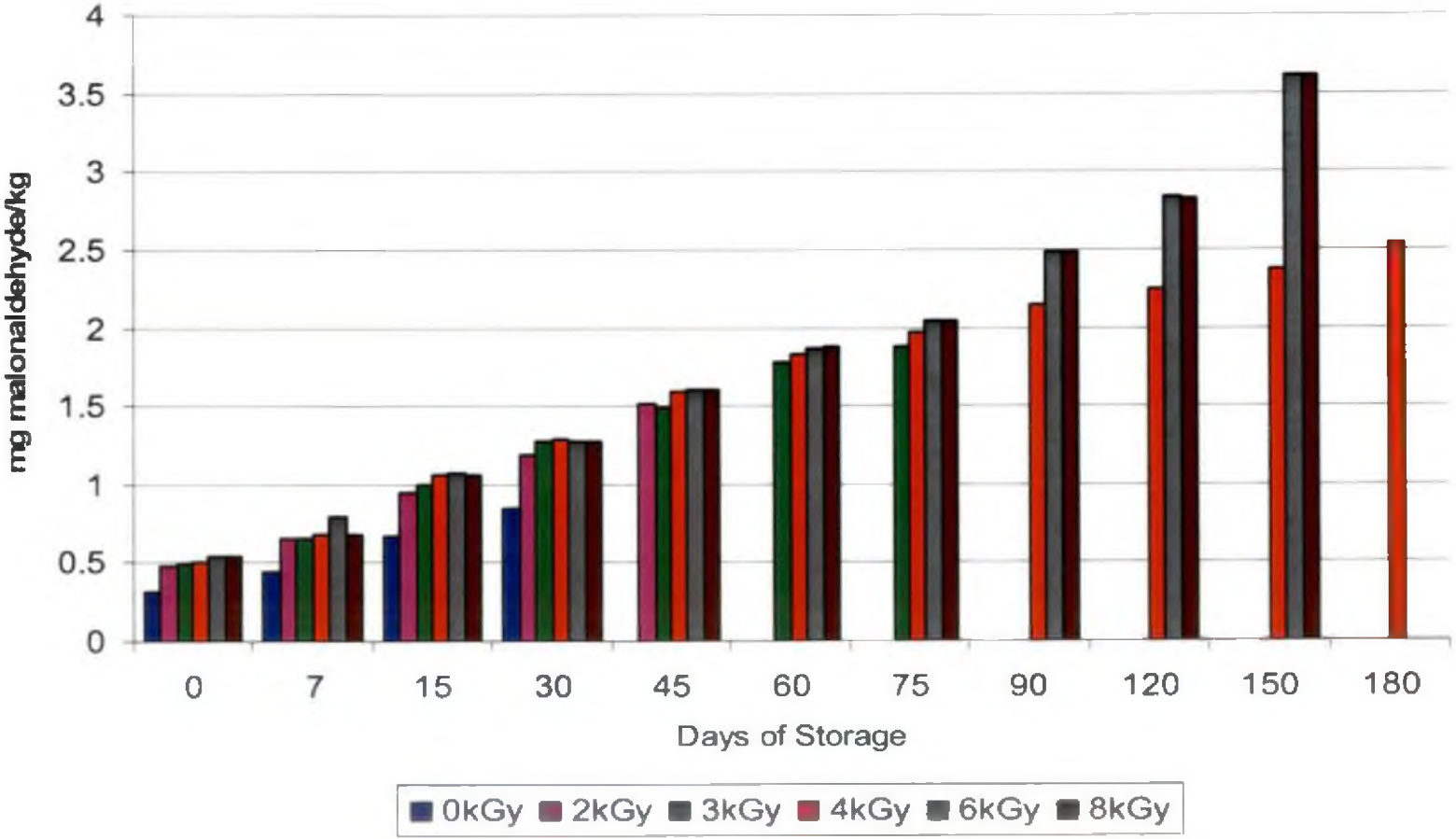


Figure3.TBARS value of 15 per cent moisture level dog biscuit

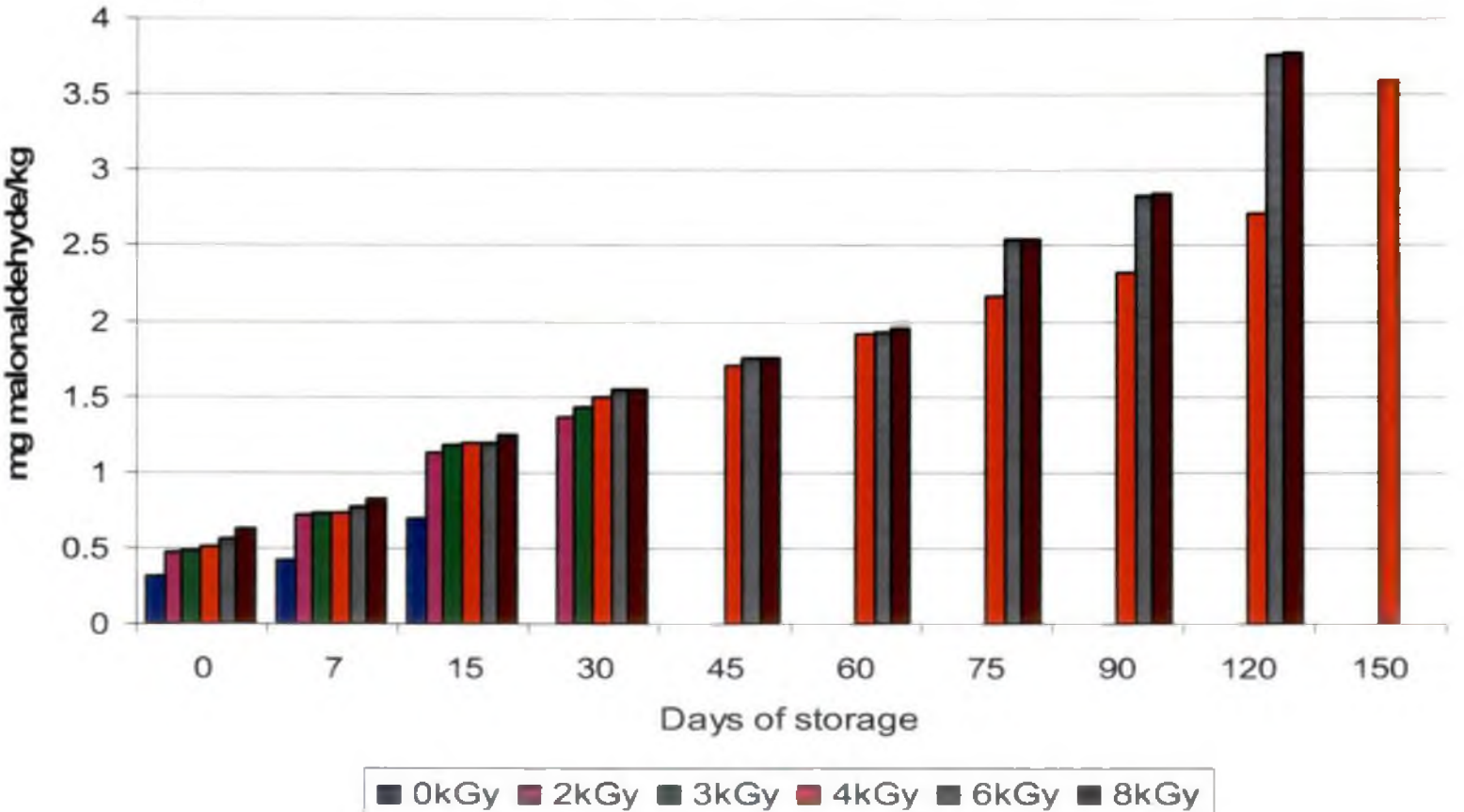


Figure 4 TBARS values of 25 percent moisture level dog biscuit

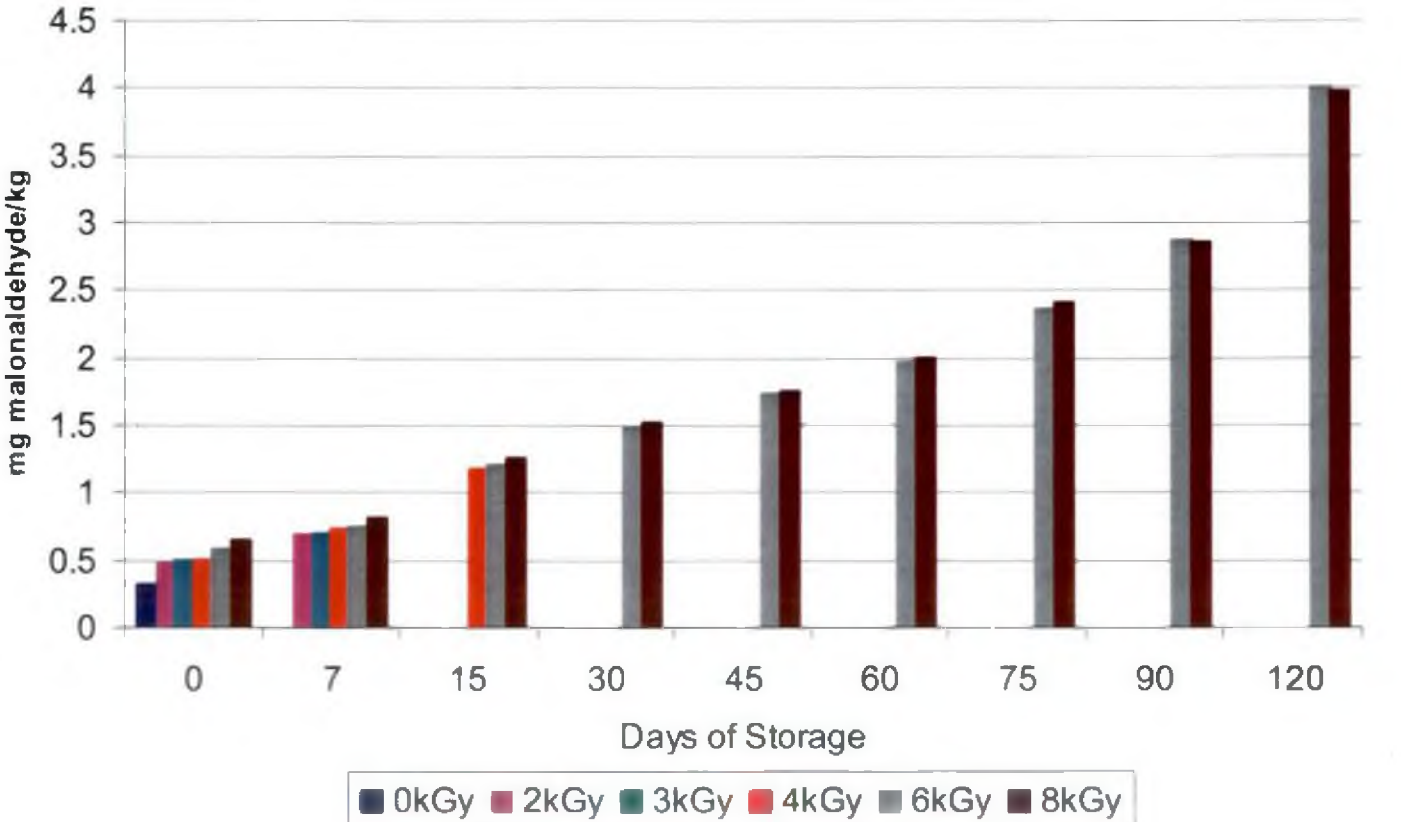


Figure 5 Growth trend of APC in10 per cent moisture level dog biscuit

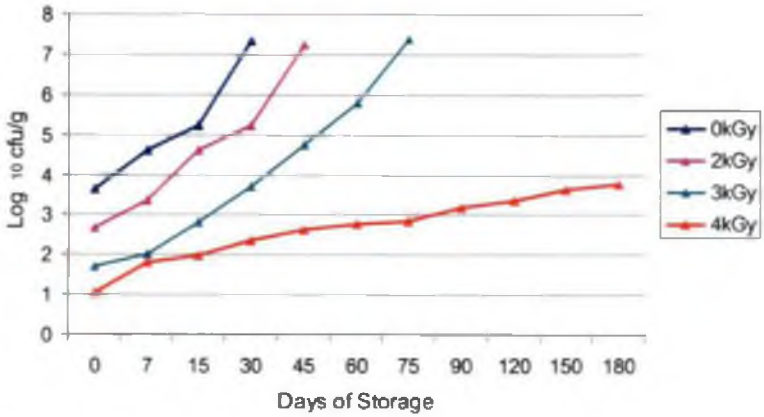


Figure 7 Growth trend of APC of 25 per cent moisture level dog biscuit

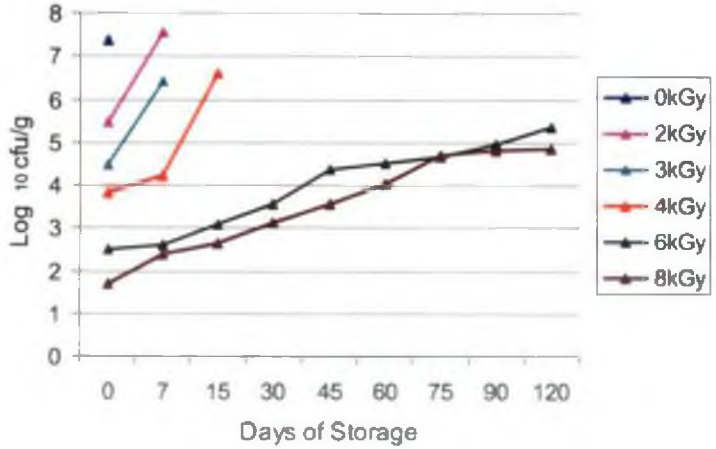


Figure 6 Growth trend of APC in 15 per cent moisture level dog biscuit

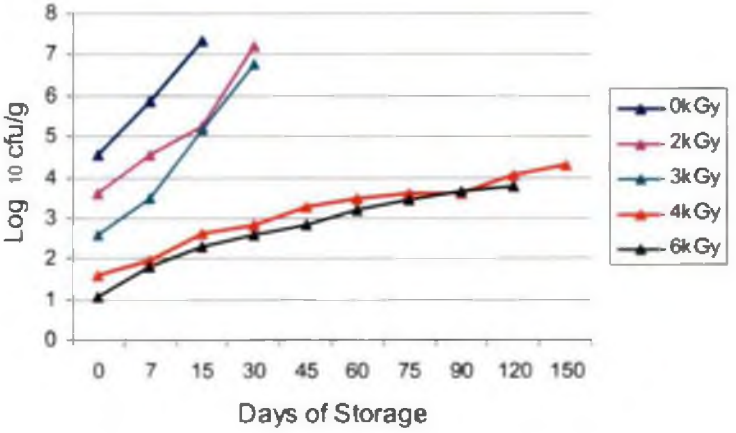


Figure 8 Growth trend of Yeast and mould count in 10 per cent moisture level dog biscuit

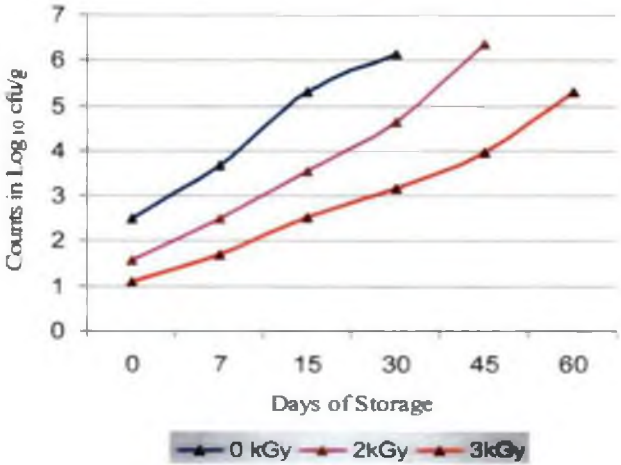


Figure 10 Growth trend of Yeast and mould count in 25 per cent moisture level dog biscuit

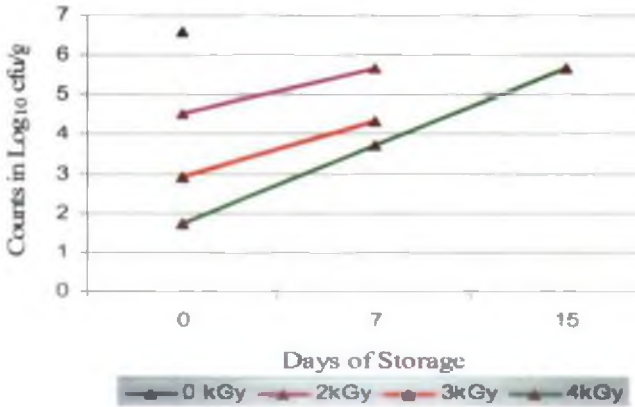


Figure 9. Growth trend of Yeast and mould growth in 15 per cent moisture level dog biscuit

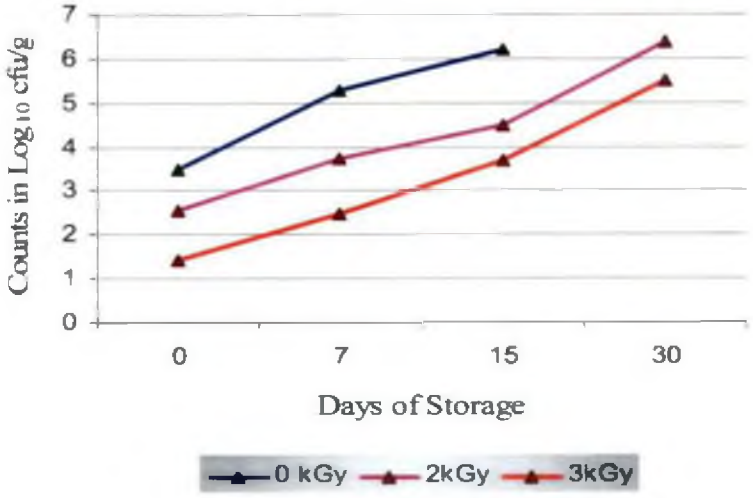
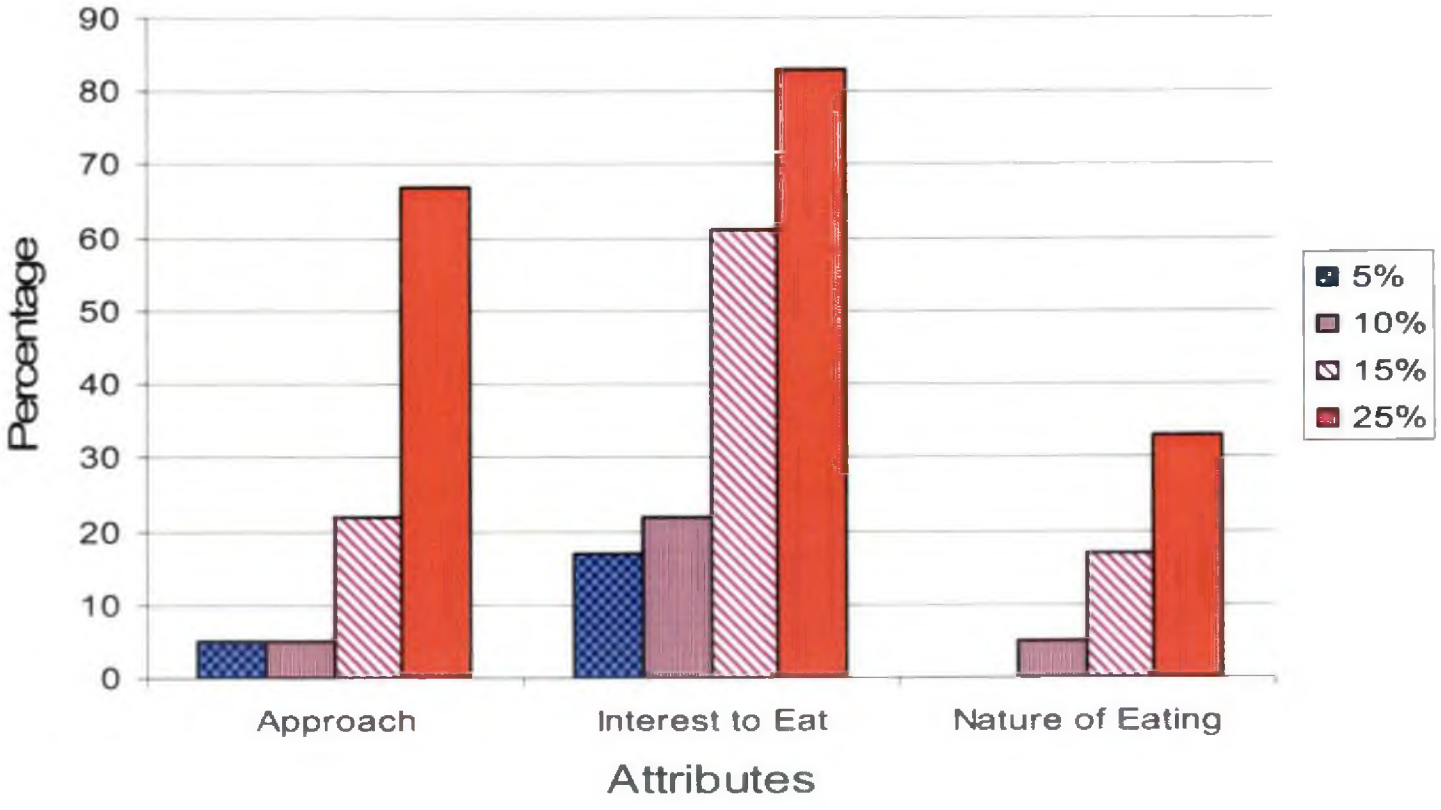


Figure11. Graphical representation of different attributes from score card of high category



Discussion

DISCUSSION

Dog biscuits were prepared and baked at 200°C in order to attain a moisture level of approximately 10, 15 and 25 per cent and packed in HDPE packets of 200g each. These biscuits were irradiated at different doses of zero, 2.0, 3.0, 4.0, 6.0 and 8.0 kGY. The biscuits containing 5 per cent moisture were used for feeding studies in pet dogs. The irradiated and control samples were stored till indications of spoilage were noticed visually and organoleptically or up to 180 days which ever was earlier. Various parameters such as proximate compositions, calcium and phosphorus content, TBARS values, microbiological load and palatability/ acceptability attributes were analysed during the storage period.

5.1. PROXIMATE COMPOSITION

National Research Council (1985) divided dog biscuits in to three categories depending on their moisture content. The actual moisture content of three groups of dog biscuits prepared and the effect of different doses of irradiation, *viz.*, 0, 2.0, 3.0, 4.0, 6.0 and 8.0 kGy during the storage period were analysed. Depending on the level of moisture the percentage of various proximate compositions like ether extract, protein content, crude fibre, ash, NFE, GE content, calcium and phosphorus contents were also varied. The moisture contents in all the samples were less than that of NRC (1985) requirements, where the semi moist dog biscuits can have 25 to 30 percent moisture. The various doses of irradiation have not significantly affected the proximate composition including calcium and phosphorus content of dog biscuits of all moisture levels. Urlings *et al.* (1993) reported that slaughter house byproducts are good source of protein, minerals and vitamins for pets. The dog biscuits prepared in the present study were having a

good percentage of slaughter house byproducts in the form of meat cum bone meal, blood and rendered fat. Hence all the samples prepared had sufficient quantity of fat, protein, ash, calcium and phosphorus. These proximate compositions including calcium and phosphorus content were unaffected even at the irradiation dose of 8.0 kGy. Wheeler *et al.* (1991) reported that the proximate compositions of ground beef patties were not affected by irradiation.

The storage of irradiated biscuits showed varying degrees of keeping qualities depending on the moisture content and the irradiation doses. During the storage period there was no significant difference with respect to the proximate compositions including calcium and phosphorus content. Wheeler *et al.* (1991) has not found any significant difference in proximate compositions between irradiated ground beef patties up to 5 weeks in chiller storage. Marathe *et al.* (2002) also observed that irradiation has got no effect on the proximate compositions in the case of wheat flour in storage up to 6 months at ambient temperature.

It was observed that as the percentage of moisture increased the GE content of the samples reduced proportionately due to high water content in the final product. Neither the dose of irradiation nor the days of storage had any significant effect on the proximate composition including calcium and phosphorus content of dog biscuits. The moisture percentage and the dose of irradiation had significant influence on the keeping quality with a synergistic effect of low moisture and higher dose of irradiation.

Grolichova *et al.* (2004) reported that ionizing radiation can be used for sterilization of diets for animals and opined that ionizing radiation reduces the risk of contamination originating in animal herd. DeRouchey *et al.* (2003b) reported that the irradiated feed stuffs had no impact on pig performance compared with non irradiated diets. As the dog biscuits treated at different doses of irradiation had

shown no effect on their compositions, it can be effectively utilized for preserving intermediary moisture dog foods which has got more palatability.

5.2. THIOBARBITURIC ACID REACTING SUBSTANCES

The TBARS values are the indication of development of oxidative rancidity in stored food items. It was observed a significant ($P<0.05$) increase due to irradiation on the day of preparation and as storage days increased. In the absence of any change in the nutritional values as reported by Eggum (1979), the brought out changes can be controlled in terms of rancidity by proper packaging conditions (Luchsinger *et al.,* 1996). Ahn *et al.* (1998) also reported irradiation accelerated lipid oxidation in case of raw meat. In present study storage period had significantly ($P<0.05$) increased TBARS values and the findings are in agreement with Ahn *et al* (2000a), Ahn *et al.* (2000b) and Badr (2004).

The low dose irradiation had showed comparatively low increase in TBARS values where as high dose irradiation led to significantly ($P<0.05$) higher values. The products irradiated at 6.0 and 8.0 kGy spoiled due to rancidity between 120 to 150 days of storage whereas the samples irradiated at 4.0 kGy had got a higher keeping quality.

Fat on exposure to high temperature and high light leads to rancidity changes which is measured as TBARS values. In the process of irradiation the product is subjected to increased temperature and light. Ahn *et al.* (1998) reported that exposure to oxygen is more important factor than irradiation in catalysing lipid oxidation. Ahn *et al.* (1998), Ahn *et al.* (2000a) and Ahn *et al.* (2000b) concluded that irradiation had got no effect in the production of volatiles due to lipid oxidation. The authors were of the opinion that exclusion of air or packaging under anaerobic conditions would prevent the bad effects of lipid oxidation. In the present study storage and irradiation had led to increased TBARS values and the samples

were packed in oxygen permeable HDPE packets might be the reason to get sufficiently ($P<0.05$) higher values compared to the day of preparation.

5.3. MICROBIOLOGICAL ANALYSIS

5.3.1. Aerobic Plate Count

The effect of irradiation at various doses is shown in Table29, Table 30 and Table 31. It was observed that in all the three moisture levels, the irradiation treatment had a significant ($P<0.05$) effect on APC. In 10 per cent moisture level irradiation at 2.0, 3.0 and 4.0 kGy significantly ($P<0.05$) reduced the initial aerobic count while, 6.0 and 8.0 kGy totally destroyed the aerobic organisms. At 25 per cent moisture level even 8 kGy irradiation reduced the APC by 78 per cent which indicates the effect of irradiation was synergised by the heating time in order to make the biscuit at lower moisture level. Sharma *et al.* (1989) in spices, Naik *et al.* (1993) in buffalo meat, Mcateer *et al.* (1995) in ready meal, Gamge *et al.* (1997) in ground beef, Murano *et al.* (1998) in ground beef Badr (2004) in meat and Kuttinarayanan *et al.* (2005) in turkey breast reported varying levels of reduction of APC due to the irradiation treatment. In the present study 2.0 kGy treatment of 10 per cent moisture level biscuit reported one $\log_{10}$ reduction and 6.0 and 8.0 kGy 100 per cent reduction in aerobic organisms.

5.3.2. Coliforms and *E. coli*

Coliform organisms were noticed only in non irradiated samples and samples irradiated at 2.0 and 3.0 kGy of 25 per cent moisture samples on the day of preparation and 7^{th} day in case of R2 and R3 samples (Table 32). In all other samples, during its storage period, coliforms and *E. coli* were not detected which clearly indicated that baking as well as irradiation had totally destroyed the organisms. Lewis *et al.* (2002) reported a complete elimination of coliforms from

poultry meat by 1.0 kGy treatment and at 2.5 kGy treatment for meat samples by Kanatt *et al.* (2005). Here in the treatment of dog biscuits containing 25 per cent moisture level the reduction was only up to 22 per cent for2.0 kGy and 62 per cent for 3.0 kGy treated groups.

5.3.3. Salmonella

The dog biscuits containing various moisture levels both control and irradiated did not reveal any salmonella organisms through out the study. Katusin-Razem *et al.* (1992) observed 10^3 reduction of these organisms in egg products by 2.5 kGy. Zhu *et al.* (2004) stated that irradiation was the most effective way to eliminate most dreadful pathogens including salmonella. Since the control sample had not shown any salmonella the effect of irradiation in the present study has to be taken in accordance with the other organisms. Normally various ingredients including animal byproducts are heavily contaminated with salmonella (Loken *et al.*, 1968) and hence irradiation at different doses will help to produce salmonella free pet foods.

5.3.4. Staphylococcus

The recovery pattern of staphylococcus followed a similar trend as that of coliforms. Other than 25 per cent moisture level samples (control, 2.0 kGy and 3.0 kGy) all other samples were free of staphylococcus. It was observed about 46 per cent reduction in case of 2.0 kGy treatment and 61 per cent in case of 3.0 kGy treatment in 25 per cent moisture level dog biscuits. As storage period increased from zero to 7 days the organisms multiplied significantly. Furkas (1998) reported radiation treatment at 2.0 to 7.0 kGy could eliminate potentially pathogenic bacteria including staphylococcus in suspected food items, Lamp *et al.* (2002) also reported a 100 per cent reduction in these organisms by 5.9 kGy treatment in sandwiches. In the present study, the dog biscuits containing 25 per cent moisture levels were

made staphylococcus free by 4.0 kGy treatment. 2.0 kGy treatment of beef fry has reduced the staphylococcus content by 94 to 98 per cent (Kuttinarayanan *et al.*, 2006b). But in the present study the reduction was only up to 46 per cent. Increased reduction may be due to increased spices and condiments present in the beef fry. Treatment of dog biscuits by 4.0 kGy will definitely make the product staphylococcus free and hence giving a shelf life of beyond 180 days.

5.3.5. Clostridium

The important spore forming anaerobic organisms leading to food poisoning, like *Clostridium perfringens* can be eliminated by gamma irradiation at various dosages. In the present study none of the samples on various stages of storage and production revealed any clostridium organisms. Gombas and Gomez (1978) observed high heat followed by gamma irradiation had an additive effect in inactivation of *Clostridium perfringens* spores. Smith and Pillai (2004) reported irradiation as the best method to decontaminate food materials especially from clostridium organisms. Since dog biscuits were prepared from slaughter house wastes and other various ingredients, there was every chance of contamination of the final product by these organisms. In order to make the dog diets free of clostridia irradiation can be effectively employed.

5.3.6. Yeast and Mould

The yeast and mould count showed a higher resistance towards irradiation with respect to various treatments. In the case of 10 and 15 per cent moisture levels 4.0, 6.0 and 8.0 kGy treatments completely destroyed yeast and mould where as 2.0 and 3.0 kGy significantly ($P<0.05$) reduced initial count. In 25 per cent moisture level dog biscuits irradiated only at doses of 6.0 and 8.0 kGy were able to destroy these organisms totally. There was a significant ($P<0.05$) difference between the treatments on various days of storage and the percentage of reduction was mainly

depending on the percentage of moisture in the dog biscuits. Prado *et al.* (2003) observed that irradiation by 10.0 kGy completely inhibited the growth of moulds in peanut samples, Rizk and Moussa (2006) observed that irradiation treatment of 4.0 kGy was sufficient to eliminate most of the fungal organisms in sugar beet seeds. Balamatsia *et al.* (2006) reported that2.0 kGy treatment completely eliminated the yeast and mould in chicken meat. In the present study, only 4.0 to 8.0 kGy treatments destroyed complete fungal population in the dog biscuits. Here the duration of baking had an added advantage in reducing the fungal population. Beef fry which contained higher percentage of moisture when irradiated at 2.0 kGy about 97 per cent reduction in yeast and mould count were noticed by Kuttinarayanan *et al.* (2006b) were the probable reason might be the presence of spices and condiments in the value added beef product. It can be concluded that if the moisture percentage is sufficiently reduced dog biscuits can be stored for a sufficient duration. By maintaining the moisture at 10 per cent level and irradiating at 4.0 kGy yeast and mould growth can be completely controlled in dog biscuits.

5.4. PALATABILITY

Dog biscuits were prepared and the moisture levels were adjusted by appropriate baking techniques and all these were irradiated at 4.0 kGy, since 4.0 kGy treated samples had a maximum shelf life. The dog owners were briefed about the palatability/ acceptability studies that to be carried out in their dogs. Eighteen dogs were selected comprising of 9 heavy breeds and 9 small or toy breeds with the help of the score card. The palatability/ acceptability of these dogs towards various moisture level dog biscuits were assessed. The total score was categorised as high, medium and low. In moisture level 5 and 10 per cent it was observed maximum percentage in medium score for various attributes both scored by the dog owners as well as the behavior of the animals. As the percentage of moisture increased dogs accepted the food very much and changed to higher score.

Wheeler *et al.* (1999) observed no significant difference in sensory qualities between irradiated and non irradiated samples. Araujo and Milgram (2004) stated that semi moist pet foods were preferred more by dogs compared to dry pet foods. The present study also indicated that 15 and 25 per cent moisture samples were preferred by dogs over 10 and 5 per cent moisture samples.

It was observed from the proximate analysis that dog biscuits containing high moisture levels will have a comparatively low percentage of nutrients. Since the dog consumes more quantity, considering their interest to eat, the low nutrient quantity can be compensated. Since it contained more quantity moisture the producer will also have more profit.

Among the dog biscuits prepared containing various levels of moisture and decontaminated by the process of gamma irradiation, it was observed that the dog biscuit containing 10 per cent moisture levels and irradiated at 4.0 kGy had a better keeping quality of more than 180 days with out any visible spoilage. From the palatability study it was observed that dog biscuits containing higher level of moisture were better preferred by dogs. Irradiation at various doses significantly reduced the bacterial load with out affecting the nutritive content of the product.

Summary

SUMMARY

In the micro family setup in many developed and developing countries pets are considered as their integral part. These pets are reared with utmost care. The commonly available pet foods in the market are of low moisture content. It is comparatively lesser palatable. If the food is of intermediary moisture content of 20 to 30 per cent, it will enhance the palatability. But it becomes more vulnerable to bacterial and fungal spoilage. In order to overcome this disadvantage irradiation technology can be used. FDA approved irradiation dose of 25 kGy (max.) for animal feed and pet food for decontamination in 1997. Therefore, this study is aimed at increasing the keeping quality of intermediary moisture pet food prepared using slaughter house byproducts which is more palatable than low moisture pet food by employing gamma irradiation. This study will also help to introduce a new technology by which shelf-stable pet food can be manufactured utilising the underutilised slaughter house byproducts which would enable the meat industry more economically viable.

The most popular and fast moving pet food *i.e.,* dog biscuits were prepared using meat cum bone meal, plain flour (*Maida*), blood, rendered fat, eggs, wheat bran and black gram flour. The mix was baked at different time-temperature combinations to attain different moisture levels (10 per cent, 15 per cent and 25 per cent). These were packed in High Density Polyethylene (HDPE) packets of 200g each and subjected to gamma irradiation at different doses of zero, 2.0, 3.0, 4.0, 6.0 and 8.0 kGy using Gamma Chamber 5000. The irradiated and non irradiated control samples were analysed for various quality parameters, *viz.,* proximate composition, development of rancidity, microbiological quality and palatability attributes on the day of preparation and then on 7, 15, 30, 45, 60, 75,

90, 120, 150 and 180 days of storage at ambient temperature or till spoilage, which ever was earlier.

The proximate compositions like moisture content, ether extract, protein content, crude fibre, ash, nitrogen free extract, gross energy content, calcium and phosphorus content were analysed. Irradiation at different doses did not significantly affect any of the above proximate compositions. The control samples and the irradiated samples of the same moisture level were shown a non significant difference in the proximate compositions as the days of storage increased. But as the moisture content of the dog biscuits increased all the other proximate compositions were reduced proportionately due to the high water content in the final product.

The TBARS values are the indication of rancidity changes in the stored food items. It was observed a significant ($P<0.05$) difference in TBARS values in control and irradiated samples. Corresponding to the increase in the irradiation dose and the days of storage, the TBARS values were increased significantly ($P<0.05$). The samples irradiated at 6.0 and 8.0 kGy of all the moisture level dog biscuits were spoiled due to rancidity between 120 to 150 days of storage. But the dog biscuits irradiated at 4.0 kGy had a higher keeping quality with respect to rancidity than other samples.

The samples have shown a significant ($P<0.05$) decrease in aerobic plate counts as the dose of irradiation increased. In 10 per cent moisture level dog biscuits, the samples irradiated at 6.0 and 8.0 kGy revealed 100 per cent reduction of aerobic organisms where as it was at 8.0 kGy in case of 15 per cent moisture level. There was a gradual and significant ($P<0.05$) increase noticed in all the dog biscuits with respect to aerobic plate count as storage days increased.

Coliform organisms were present only in the 25 per cent moisture dog biscuits and which were completely destroyed at irradiation dose of 4.0 kGy and above. There was a gradual and significant ($P<0.05$) reduction in coliforms noticed in dog biscuits as the irradiation dose increased. All the dog biscuits in spite of their moisture contents and irradiation levels revealed no *E coli* organisms.

Staphylococcal organisms also showed the same pattern as that of the coliforms. They were only present in the dog biscuits of 25 per cent moisture level. Irradiation dose of 2.0 kGy produced a 46 per cent reduction in the counts and 4.0 kGy revealed total destruction of the organisms from the food. The 10 and 15 per cent moisture level samples were free from staphylococcal organisms.

All the samples were free from Salmonella, Clostridium and faecal streptococcal organisms through out the study period.

In the samples of 10 and 15 per cent moisture level the yeast and mould were completely destroyed at 4.0 kGy and above while the same result was obtained at 6.0 kGy and above for 25 per cent level samples. Irradiation doses had shown a significant ($P<0.05$) reduction in yeast and mould counts in all the samples under study. The storage days have shown significant ($P<0.05$) effect on the increase of the yeast and mould counts in all moisture level dog biscuits.

For palatability/acceptability studies, dog biscuits were prepared at four different moisture levels of 5, 10, 15 and 25 per cent and irradiated at 4.0 kGy since 4.0 kGy treated samples had a maximum shelf life. The dog biscuits were given to 18 different dogs and the responses were collected in the form of a score card. The dogs preferred the intermediary moisture level dog biscuits of 15 and 25 per cent moisture level than the dry dog biscuits of 5 and 10 per cent moisture levels. The study revealed that the dogs got a higher affinity towards high moisture content food than the low moisture level/dry food.

It was observed from the above results that the dog biscuits containing 10 per cent moisture level and irradiated at 4.0 kGy had got better keeping quality of more than 180 days. The various irradiation doses have not significantly affected the proximate composition although it reduced the bacterial load significantly. Irradiation at higher doses significantly increased the development of rancidity in the dog biscuits. Finally the palatability/acceptability studies revealed that high moisture pet foods are better preferred by the dogs.

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THE EFFECT OF LOW DOSE GAMMA RADIATION ON THE QUALITY OF INTERMEDIARY MOISTURE PET FOOD

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ABSTRACT

The effect of low dose gamma irradiation on the quality of intermediary moisture pet food was assessed in the present study. Dog biscuits were prepared using meat cum bone meal, plain flour (*Maida*), blood, rendered fat, eggs, wheat bran and black gram flour. The mix was baked at different time temperature combinations to attain three different moisture levels of 10, 15 and 25 per cent. The samples were separately packed in High Density Polyethylene (HDPE) packets at a rate of 200g each and then subjected to gamma irradiation using Gamma Chamber 5000 at various doses of zero, 2.0 kGy, 3.0 kGy, 4.0 kGy, 6.0 kGy and 8.0 kGy. After irradiation the samples were kept in ambient temperature for further analysis like proximate composition, development of rancidity, microbiological quality and palatability/ acceptability attributes on the day of preparation and then on 7, 15, 30, 45, 60, 75, 90, 120, 150 and 180 days of storage or till spoilage which ever was earlier.

The proximate compositions of the dog biscuits of all moisture levels were not significantly affected by various doses of irradiation and days of storage.

The TBARS values were shown a significant ($P<0.05$) increase with the increase in the doses of irradiation. The days of storage were also affected the TBARS values significantly ($P<0.05$). The samples irradiated at 6.0 and 8.0 kGy of all moisture level dog biscuits were spoiled between 120 to 150 days where as the samples irradiated at 4.0 kGy showed a higher shelf life in the absence of any developed rancidity changes.

The aerobic plate count was shown a significant reduction as the dose of irradiation increased, also a significant ($P<0.05$) increase was noticed with the days

of storage. In 10 per cent moisture level dog biscuits, the samples irradiated at 6.0 and 8.0 kGy revealed 100 per cent reduction of aerobic organisms, where as the same result was obtained for the samples irradiated at 8.0 kGy in case of 15 per cent moisture level dog biscuits. Coliforms and staphylococcal organisms were present only in 25 per cent moisture level dog biscuits and they were completely destroyed at an irradiation dose of 4.0 kGy and above. All the samples under study were found free from E. coli, Salmonella, Clostridium and faecal streptococcal organisms. The yeast and mould count also showed the same trend as aerobic organisms. The samples of 10 and 15 per cent moisture level dog biscuits were found free from yeast and mould when irradiated at 4.0 kGy and above, while the same result was obtained for 25 per cent moisture level samples on irradiation at 6.0 and 8.0 kGy.

The palatability/ acceptability studies were conducted using the dog biscuits of 5, 10, 15 and 25 per cent moisture levels irradiated at 4.0 kGy. The dogs preferred intermediary moisture dog biscuits of 15 and 25 per cent moisture levels when compared with dry dog biscuits having 5 and 10 per cent moisture.

It was observed that gamma irradiation of dog biscuits significantly reduced the microbial load with out affecting their proximate compositions. But higher doses of irradiation induced the production of oxidative rancidity. The palatability studies revealed that intermediary moisture level food was more preferred by the dogs than dry food. This study also found that the dog biscuits containing 10 per cent moisture and irradiated at 4.0 kGy had got the highest shelf life of more than 180 days.