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Effect of Irradiation and Curry Leaves Extract on Quality Attributes and Shelf Life of Chicken Emulsion

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ABSTRACT

The present study was conducted to evaluate the effect of irradiation and antioxidants on quality attributes and shelf life of the chicken emulsion during refrigerated storage. Four treatments evaluated include: Control (without irradiation and antioxidant), T1 (irradiation without antioxidant), T2 (butylated hydroxyl anisole–(BHA) - 0.02%) and T3 (450 ppm equivalent curry leaves (*Murrayakoenigii*) phenolics. Total phenolic contents (mg per gram of powder) as tannic acid equivalent of curry leaves was ranged from 6.78 to 7.27. There was a concentration dependent increase in reducing power (absorbance value of 0.3 at 50 μg phenols to 0.9 at 50 μg phenols) and 1,1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity (70% at 50 μg phenols to 85% at 250 μg phenols) of curry leaves extract. Highly significant differences ($P<0.05$) in pH, TBARS (Thiobarbituric acid reactive substance) values and microbial counts was observed between control and treatment groups and also between storage periods. Incorporation of curry leaves extract significantly ($P<0.05$) lowered TBARS values. During storage, all the samples showed significant ($P<0.05$) decrease in pH and an increase in TBARS values and total plate counts, psychrotropic counts and lactobacilli counts. *Escherichia coli* and Salmonella were not detected in all the treatment groups. No significant ($P>0.05$) difference was noticed in sensory attributes among the control and treatment groups up to 7 days of storage. However, the deteriorative changes were faster in control samples. Thus, the present study indicated the promising potential of irradiation as an efficient method for reducing microbial load in meat products and curry leaves extract may be used as a potential source of antioxidants to protect against oxidative rancidity.

Keywords : *BHA, Chicken emulsion, Curry leaves, Irradiation, Lipid oxidation, Natural antioxidants.*

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INTRODUCTION

Increasing urbanization, industrialization, rising income, changing food habits with preference for convenience products, rise in working women force and rapid proliferation of fast food outlets offer a tremendous potential for rapid growth of processed meat products. Moreover, in case of broiler chicken, processing of meat into further processed and value added meat products, leads to better utilization of surplus meat in season, thereby controls market glut of poultry supply. Among the value added meat products, sausages, patties, nuggets and other meat emulsion based snack type products are very popular worldwide, even in local markets. Many national and international fast food chains like Venkeys, Godrej, McDonald's are selling many meat emulsion based, ready-to-eat snack type products (Deogade *et al*. 2008). Similarly, the availability of the emulsion as ready to cook items like *idly/vada* batters in the market will facilitate the consumers to prepare products of their choice by means of various cooking methods and incorporating ingredients like onion, chilli and other vegetables. Products like meat croquettes, scrambled emulsion, enrobed eggs, *kebab* and some Indian delicacies could be made with the meat emulsion.

Adequate preservation technologies must be applied while converting meat into value added products, as meat is a rich nutrient matrix that provides a suitable environment for the proliferation of microorganisms and also the meat products can easily be contaminated by microorganisms from raw materials (ground meat, binders, spices, etc.), equipment and employees. Further, processing conditions like grinding and additions of sodium chloride accelerate oxidation (Kanner *et al*. 1991).

Irradiation is one of the promising preservation technologies, where food is exposed to a carefully controlled amount of energy in the form of high speed particles or rays. More than 100 years of research that have gone into understanding of the harmless and effective use of irradiation and currently, more than 50 countries have given approval for over 60 foodstuffs to be irradiated (Scott and Suresh 2004). Exposure of meat to ionizing radiation, however, results in lipid oxidation due to the formation of radiolytic products from free radicals that are formed during treatment. Therefore, interest is growing in identifying new cost effective natural antioxidants that could serve as alternatives to the synthetic compounds. Curry leaves (*Murraya koenigii*) are native from East Asian countries and are commonly used to flavour a range of dishes. Monoterpene derived hydrocarbons and alcohols in the extracts are recognized for their efficacy in providing significant antioxidant activity to foods (Ningappa *et al*.2008). With this

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background, the present study was undertaken to evaluate the effect of irradiation and antioxidants on physico-chemical, microbiological and sensory qualities of chicken emulsion.

MATERIAL AND METHODS

Materials: Fresh broiler chicken meat was procured separately for each of the five replications on different occasions from the local retail shops and utilized for this study. The fat, skin, gizzard and heart were also collected along with the meat. After removing the tendon and other connective tissues, meat and byproducts were chilled overnight at 4±1°C and frozen and stored at -20°C until use. Butylated hydroxyl anisole (BHA), 2-thiobarbituric acid, trichloroacetic acid (Merk, Mumbai, India), tannic acid, 1,1-diphenyl 2-picrylhydrazyl (DPPH) (Sigma–Aldrich, New Delhi, India) used in the study were of analytical grade. Peptone, plate count agar, MRS, Eosine and methylene blue agar and Salmonella agar were procured from Himedia, Mumbai, India.

Curry leaves extract (CLE) : Fresh curry leaves (*Murraya*) obtained from local market was washed well with water to remove the adhering dust. They were air dried and ground into powder in a heavy duty grinder (Soni appliances, Mumbai, India) and sieved using a 60 mesh sieve and packed and stored at room temperature in high density polyethylene bags until extraction. About 20 gm of dried powder was mixed with 100 ml boiled distilled water and left for 5 min. The extract was obtained by filtration through No.1 Whatman filter paper and analyzed for pH, total phenolic content (Escarpa and Gonzalez 2001), reducing power (Oyaizu 1986) and DPPH radical scavenging activity (Singh *et al*. 2002). Freshly prepared extract was used for each replication on the basis of phenolic

Preparation of chicken emulsion: The frozen chicken meat samples were thawed overnight and cut into thin slices (Sirman, Italy) and minced twice (13 mm plate followed by 6 mm plate) using a meat mincer (Sirman, Italy).The chicken emulsion was prepared by fine chopping the optimum levels of ingredients [deboned chicken meat- 60%, byproducts (skin, gizzard heart and fat)-20%, non-meat ingredients (binder, condiments, spice, etc.)-20%] in bowl chopper (Model X70, Scharfen, 58413 Witten, Germany) and this emulsion was divided into four lots. One lot was kept as such and served as the control (without irradiated and antioxidant). Second lot was kept without adding antioxidant and irradiated (T1). Third lot was treated with antioxidant butylated hydroxyl anisole (BHA) (0.02%)and irradiated (T2). Fourth lot was incorporated with curry leaves (*Murraya koenigii*) extract (450 ppm equivalent phenolics) and irradiated(T3). The volume of extract added was replaced with distilled water in control and other treatment group samples. The BHA was dissolved in 5 ml vegetable oil before addition and the equal quantity of oil was added to other samples to maintain uniformity. After adding curry leaves extract/BHA, respective emulsion samples were thoroughly hand mixed for 5 min to ensure uniform mixing. From each lot, 6 packs of emulsion (200 gm each) were individually vacuum packed in sterile, low density polyethylene bags (700 gauge; Water vapour transmission rate 0.4 g/m^2/day; Oxygen transmission rate 1,800 mL/m^2/day).The prepackaged meat emulsion samples, other than control were irradiated at a dose rate of 3 kGy in package irradiator (Gamma Chamber 5000) with a ^{60}Co source, in irradiation unit at ANGR Agriculture University, Hyderabad. Temperature of emulsion samples, before and after irradiation was maintained at 4±1°C by covering the packages with frozen ice gels in an insulated box. All samples were then stored at refrigeration temperature (4±1°C) up to 35 days. Samples drawn periodically at weekly intervals on 1, 7, 14, 21, 28 and 35 day of storage for evaluation of physico-chemical and microbial quality attributes and product prepared from emulsion was subjected to sensory evaluation.

Estimation of antioxidant properties of curry leaves extracts (CLE)

Total phenolics: The concentration of total phenolics in CLE was determined by the Folin–Ciocalteus (F–C) assay (Escarpa and Gonzalez 2001). Suitable aliquots of curry leaves extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F–C (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance recorded at 725 nm (Shimadzu, Japan) after 40 min. The total phenolics content was calculated using tannic acid as standard and results were expressed as μg tannic acid equivalent.

1,1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity: The ability of CLE to scavenge DPPH radical was determined as per the method of Singh *et al*. (2002). The CLE (50 and 100 mg phenolics) diluted with 0.1 M Tris–HCl buffer (pH 7.4) was mixed with 1 ml of DPPH (250 mM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and the absorbance was measured at 517 nm (Shimadzu, Japan). The scavenging activity was quantified by the following equation:

$$\text{Scavenging activity \%} = (\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Blank}} \times 100$$

Reducing power: The method described by Oyaizu (1986) was employed for determining the reducing power. Ten milligram phenolics from CLE were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and incubated with 2.5 ml potassium ferricyanide (1% w/v) at 50 ^{0}C for 20 min. At the end of incubation, 2.5 ml of 10% trichloroacetic acid solution was added and centrifuged at 3000 gm for 10 min. The supernatant was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride

(0.1% w/v) solution. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the sample.

Assessing quality characteristics of chicken emulsion

Proximate composition: The percent moisture content was determined by hot air oven drying, protein by automatic Kjeldhal method, fat by Soxhlet extraction with petroleum ether and total ash by Muffle furnace as described in AOAC (1994).

pH: The pH of the emulsion was determined by following the procedure of Jay (1992). Meat emulsion sample weighing 25 gm was blended with 100 ml of distilled water for one minute in a homogenizer (Model Miccra D8-Si, ART Moderne Labortechnik, Germany). From the total homogenate, a 50 ml aliquot portion was immediately used for determination of pH using digital pH meter (Model 420A+, Thermo Orion, USA) after standardization of the instrument with two standard buffers (pH 4 and pH 14).

2-Thiobarbituric acid reactive substance (TBARS) value (mg malonaldehyde/kg): The TBARS value of the sample was determined based on the procedure of Witte *et al*. (1970). Trichloroacetic acid (TCA) extract of the sample was prepared by homogenizing 4 gm emulsion in 20 ml 20% TCA solution (20 % TCA in 2M phosphoric acid). The extraction slurry was centrifuged at 5500 rpm for 15 min. The contents were filtered through No.1 Whatman filter paper to get the TCA extract. Then 3 ml of TCA extract was mixed with 3 ml of 2-thiobarbituric acid (0.01%) reagent in stoppered flask, and boiled for 35 minutes in boiling water bath. A blank was also prepared by heating 3 ml of 2- thiobarbituric acid reagent with 3 ml of 20% TCA in boiling water bath. The sample was cooled and absorbance was measured at 532 nm using UV-VIS spectrophotometer (Shimadzu, Japan) and the TBARS values were calculated using a TBA standard curve and expressed in mg malonaldehyde/kg.

Microbial counts (log_{10} cfu/gm): The total plate counts, psychrotropic counts, *E.coli* count and *Salmonella* counts per gram of chicken meat emulsion at refrigerated temperature were estimated as per the procedure recommended by Chestnut *et al.* (1977). Ten grams of sample was thoroughly blended with 90 ml of 0.1% sterile peptone water for 2 min. One ml sample was aseptically pipetted out into tubes containing 9 ml of 0.1% peptone water. Serial dilutions of the sample were prepared and the samples in duplicates were inoculated by pour plate method on standard plate count agar for enumeration of total plate counts (TPC) and psychrotropic count (PPC). Plates were incubated at 37 ^{0}C for 48 h for APC and 7 ^{0}C for 10 days for PPC. After specified incubation period, the plates revealing colony forming units were selected and the plate counts were expressed as log_{10} numbers per gram. For estimation of *Lactobacillus*,*E.coli* count and *Salmonella* counts, the procedure followed was same as the above, except that selective and differential media MRS, Eosine and methylene blue agar and Salmonella agar were used in place of standard plate count agar, respectively.

Sensory evaluation: The meat emulsion of each treatment was filled in steel moulds and cooked for 20 min using steam without pressure to an internal temperature of 80°C as indicated by the temperature probe. The cooked meat blocks, thus formed were immediately chilled and sliced into nuggets of uniform size to study sensory attributes. The sensory attributes in terms of colour and appearance, flavor, juiciness, texture and overall acceptability of chicken nuggets were evaluated using a8 point hedonic scale(8, extremely desirable; 1, extremely undesirable) by an experienced panel. The sensory evaluation was discontinued whenever the total plate count of chicken emulsion samples exceeded the maximum acceptable level of 7 log_{10}value.

Statistical analysis: The data was analyzed using General Linear Model procedure of statistical package for social sciences (SPSS) 15.0 version and comparison of means tested using Duncan's multiple range test outlined by Snedecor and Cochran (1967) and significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Antioxidant properties of curry leaves extract: Total phenolic content (mg per gram of powder) as tannic acid equivalent of curry leaves was ranged from 6.78 to 7.27. Biswas *et al.* (2012) used solvents like ethanol, hot water, ethanol and water and reported that the total phenolic content of curry leaves as Gallic acid equivalent ranging from 2.7 to 3.7 mg/gm. However, Ningappa *et al.* (2008) reported polyphenolic content (mg GAE/g curry leaf extract) of water extracts of curry leaves was about 54 mg/gm extract. This could be due to variation in the concentration due to geographical location and extraction procedure.

The DPPH is used as a free radical to evaluate the antioxidant activity present in natural sources. The DPPH radical scavenging activity was around 70% at 50 μg phenols and increased with increasing phenolic content. Ningappa *et al.* (2008) reported that the water extracts of curry leaves exhibited DPPH radical scavenging activity of 41 % at 20 μg. An increase in the concentration of the plant extracts, progressively increased the radical scavenging activity. This correlates well with the concentration of total phenolic compounds per unit volume of leaves powder. Negi and Jayaprakasha (2003) and Naveena *et al.* (2008) have also reported an increase in radical scavenging activity with an increase in the concentration of pomegranate peel extract and pomegranate rind powder extract, respectively.

The reducing power of different levels of curry leaves extract (CLE) was carried out using potassium ferric anide method. The reducing properties are generally associated with the presence of reductones and the antioxidative action of reductones is based on the breaking of free radical chains by the donation of hydrogen atom (Gordon 1990). A concentration dependent increase in reducing power of CLE was noticed. A significant increase in reducing the power of the extracts of curry leaves with increase in concentration have been reported by Ningappa *et al.* (2008).

Effect of CLE and BHA on quality attributes of chicken emulsion

The moisture, protein, fat and ash content (%) of chicken emulsion were 66.19, 18.88, 12.46 and 2.48, respectively. The changes in pH of control and irradiated chicken emulsion during refrigerated storage are given in Table 1. A highly significant difference ($P<0.05$) in pH was observed between treatment groups and also between storage periods. The overall mean pH values of control meat emulsion samples were significantly ($P<0.05$) lower compared to the treated group at all storage periods studied. This could be attributed to the production of lactic acid through *Lactic acid bacteria* (LAB) metabolism for the reduction of pH (Eirini *et al.* 2008). Newton and Gill (1978) also reported that the vacuum packaging causes a microbial shift, resulting in the development of a *Lactobacillus* dominated population rather than a high spoilage potential *Pseudomonas* population. There was no significant ($P>0.05$) difference in pH among different irradiation treatments (T1, T2 and T3). The incorporation of either butylated hydroxyl anisole (BHA) or curry leaves extract (CLE) did not cause significant change in the pH of meat emulsion. This is in accordance with Das *et al*. (2011), who found no change in the pH due to addition of curry leaf powder in ground poultry and goat meat, respectively. This could be due to the fact that meat is having good pH stabilizing property. During storage, the pH values of control samples decreased significantly ($P<0.05$) from 1st day to 35th day of refrigerated storage. The out growth of dominant *lactobacillus* bacteria in the anaerobic environment and subsequent production of lactic acid could be the reason for the reduction in pH over period of storage in the control samples. Though, there was a gradual decrease in the mean values of pH of all the three irradiated chicken meat emulsions during the storage period, the changes were not significantly different. Destruction of certain *lactobacillus* bacterial population at the time of irradiation could be the reason for lesser acidity development in the treated samples.

The overall mean of TBARS values of irradiated meat emulsion samples without antioxidant (T1) were significantly higher compared to control as well as T2 and T3 (Table1). This is in accordance with Hoyland and Taylor (1991) who reported that irradiated meat showed higher TBARS values due to fat auto oxidation by free radicals produced during irradiation. The overall mean of TBARS values for BHA incorporated chicken meat emulsion (T2) was significantly ($P<0.05$) lower compared to control, T1and T3. Addition of BHA has been found to be effective in lowering lipid oxidation in T2 meat emulsion samples stored at 4º C for up to 35 days. This is in accordance with the findings of Shahidi (1998) who also found lower TBARS value in meat products containing BHA compared to products without antioxidants. The BHA is free radical terminator that has a cyclic carbon ring capable of accepting/tying a free radical molecule (Faridah 2008).

The overall mean of TBARS values of curry leaves extract incorporated meat emulsion samples showed significantly ($P<0.05$) lower when compared to T1 samples. This could be due to the antioxidant activity of phenolic compounds present in the CLE, which act as free radical scavengers. Adding phenolic antioxidants to meat is an effective method in reducing the oxidative reaction and several workers have also been reported retardation of lipid oxidation of meat products by incorporating various plant antioxidants like grape seed, green tea, pomegranate juice, drumstick (*Moringaoleifera*) leaves extract etc. (Muthukumar *et al*. 2012; Naveena *et al*. 2008). These compounds were reported to quench oxygen derived free radicals by donating a hydrogen atom or an electron to the free radical (Wanasundara and Shahidi 1998).

On storage, both control and irradiated meat products showed significant increase ($P<0.05$) in TBARS values. The emulsion meat samples irradiated without any antioxidants had significantly higher ($P<0.05$) TBARS values on storage as compared to non-irradiated control and irradiated meat products with antioxidant. A similar trend was also observed by Sweetie *et al.* (2005). The higher TBARS values of irradiated samples may be explained by the fact that auto-oxidation of fat is accelerated by free radicals produced during irradiation to form hydroperoxides, which breakdown into various decomposition products including aldehydes, of which malonaldehyde is the major TBA-reactive substance (Hoyland and Taylor 1991). A significant increase in lipid oxidation as storage time increased was also noticed in irradiated beef by Ismail *et al.* (2008).

A highly significant difference ($P<0.05$) in total plate counts, psychrotropic counts and *lactobacilli* counts were observed between the control and treatment groups as well as between storage periods (Table 1). Lower total plate count, psychrotropic counts and *lactobacilli* counts ($\log_{10}$cfu/gm) values in all the treatment groups might be due to the effect of irradiation. There are several reports on the significant effects of irradiation on reduction/elimination of bacteria (Sweetie *et al.* 2005; Eirini *et al.* 2008). There was a linear and highly significant increase

Table 1: Changes in pH, TBARS values and microbial quality of chicken emulsion due to irradiation and incorporation of curry leaves extract (CLE) and butylated hydroxyl anisole (BHA) during storage (4^{0}C).

Parameter	Treatment	Storage period (days)						Overall means (Treatments)
		1	7	14	21	28	35	
pH	Control	5.89±0.11^{Ab}	5.78±0.17^{Ab}	5.51±0.17^{ABb}	5.18±0.21^{BCb}	5.08±0.16^{BCb}	4.87±0.03^{Cb}	5.38±0.04^{p}
	T1	6.17±0.02^{a}	6.14±0.03^{a}	6.12±0.03^{a}	6.11±0.08^{a}	6.04±0.09^{a}	6.02±0.03^{a}	6.10±0.04^{q}
	T 2	6.18±0.02^{a}	6.16±0.03^{a}	6.14±0.03^{a}	6.14±0.05^{a}	6.11±0.06^{a}	6.09±0.03^{a}	6.14±0.04^{q}
	T3	6.13±0.02^{a}	6.12±0.04^{a}	6.12±0.04^{a}	6.09±0.04^{a}	6.03±0.12^{a}	6.00±0.03^{a}	6.08±0.04^{q}
	Overall means (days)	6.09±0.04^{S}	6.05±0.04^{S}	5.97±0.04^{RS}	5.88±0.04^{QR}	5.81±0.04^{PQ}	5.74±0.04^{P}	
TBARS (mg malonaldehyde/kg)	Control	0.22±0.01^{Aa}	0.44±0.02^{Bb}	0.75±0.02^{Cc}	0.87±0.02^{Db}	0.94±0.02^{Eb}	1.12±0.03^{Fb}	0.72±0.01^{q}
	T1	0.27±0.02^{Ab}	0.49±0.02^{Bc}	0.84±0.02^{Cd}	0.96±0.02^{Dc}	1.09±0.03^{Ec}	1.38±0.01^{Fc}	0.84±0.01^{r}
	T 2	0.24±0.01^{Aab}	0.38±0.02^{Ba}	0.64±0.02^{Ca}	0.75±0.02^{Da}	0.84±0.03^{Ea}	0.95±0.03^{Fa}	0.63±0.01^{p}
	T3	0.25±0.01^{Aab}	0.42±0.02^{Bab}	0.70±0.01^{Cb}	0.81±0.01^{Db}	0.95±0.02^{Eb}	1.15±0.02^{Fb}	0.71±0.01^{q}
	Overall means (days)	0.24±0.01^{P}	0.43±0.01^{Q}	0.73±0.01^{R}	0.85±0.01^{S}	0.96±0.01^{T}	1.15±0.01^{U}	
TVC (log CFU/gm)	Control	4.16±0.14^{Ab}	5.19±0.27^{Bb}	6.78±0.18^{Cb}	7.88±0.29^{Db}	9.39±0.03^{Eb}	9.64±0.02^{Eb}	7.17±0.06^{q}
	T1	2.62±0.03^{Aa}	3.27±0.02^{Ba}	4.91±0.03^{Ca}	5.93±0.04^{Da}	6.45±0.30^{Ea}	7.95±0.25^{Fa}	5.19±0.06^{p}
	T 2	2.70±0.06^{Aa}	3.25±0.02^{Ba}	4.93±0.01^{Ca}	5.90±0.07^{Da}	6.50±0.22^{Ea}	7.89±0.32^{Fa}	5.20±0.06^{p}
	T3	2.69±0.03^{Aa}	3.23±0.03^{Ba}	4.92±0.03^{Ca}	5.92±0.04^{Da}	6.67±0.22^{Ea}	7.94±0.24^{Fa}	5.23±0.06^{p}
	Overall means (days)	3.04±0.08^{P}	3.73±0.08^{Q}	5.38±0.08^{R}	6.40±0.08^{S}	7.25±0.08^{T}	8.35±0.08^{U}	
PPC (log CFU/gm)	Control	3.79±0.06^{Ab}	4.49±0.09^{Bb}	5.70±0.08^{Cb}	6.16±0.03^{Db}	6.82±0.01^{Eb}	6.95±0.01^{Eb}	5.65±0.02^{q}
	T1	2.12±0.03^{Aa}	3.27±0.02^{Ba}	3.91±0.03^{Ca}	4.11±0.03^{Da}	4.96±0.03^{Ea}	5.36±0.07^{Fa}	3.95±0.02^{p}
	T 2	2.20±0.03^{Aa}	3.29±0.03^{Ba}	3.93±0.03^{Ca}	4.14±0.05^{Da}	4.95±0.03^{Ea}	5.32±0.03^{Fa}	3.97±0.02^{p}
	T3	2.22±0.03^{Aa}	3.31±0.04^{Ba}	3.94±0.01^{Ca}	4.20±0.04^{Da}	4.99±0.05^{Ea}	5.34±0.08^{Fa}	4.00±0.02^{p}
	Overall means (days)	2.58±0.02^{P}	3.59±0.02^{Q}	4.37±0.02^{R}	4.65±0.02^{S}	5.43±0.02^{T}	5.74±0.02^{U}	
Lactobacillus (log CFU/gm)	Control	3.71±0.13^{Ab}	5.70±0.05^{Bb}	5.84±0.11^{Bb}	6.25±0.49^{BCb}	6.76±0.30^{Cb}	7.56±0.09^{Db}	5.97±0.06^{p}
	T1	2.43±0.17^{Aa}	3.26±0.03^{Ba}	3.96±0.03^{Ca}	4.46±0.09^{Da}	5.73±0.03^{Ea}	6.90±0.02^{Fa}	4.46±0.06^{q}
	T 2	2.47±0.18^{Aa}	3.32±0.05^{Ba}	3.95±0.04^{Ca}	4.41±0.09^{Da}	5.68±0.05^{Ea}	6.91±0.04^{Fa}	4.46±0.06^{q}
	T3	2.44±0.11^{Aa}	3.31±0.05^{Ba}	3.97±0.06^{Ca}	4.36±0.10^{Da}	5.73±0.07^{Ea}	6.97±0.05^{Fa}	4.46±0.06^{q}
	Overall means (days)	2.76±0.07^{P}	3.90±0.07^{Q}	4.43±0.07^{R}	4.87±0.07^{S}	5.97±0.07^{T}	7.08±0.07^{U}	

n=5. Means with different uppercase superscripts in the same row and lowercase superscripts in the same columns are significantly different (P<0.05). Control, no irradiation and no antioxidant; T1, irradiation without antioxidant; T2, irradiation with BHA (0.02%); T3, irradiation with CLE (450 ppm equivalent phenolics).TBARS-2-Thiobarbituric acid reactive substance; BHA- Butylated hydroxyl anisole; TPC- Total viable count; PPC-Psychrotropic count; CFU- Colony forming units

Table 2: Changes in sensory attributes of chicken emulsion due to irradiation and incorporation of curry leaves extract (CLE) and butylated hydroxyl anisole (BHA) during storage (4^{0}C).

Parameter	Treatment	Storage period (days)						Overall means (Treatments)
		1	7	14	21	28	35	
Appearance	Control	7.06±0.08^{E}	6.70±0.11^{DE}	5.83±0.46^{D}	4.76±0.47^{Ca}	3.16±0.45^{Ba}	1.00±0.00^{Aa}	4.75±0.09^{p}
	T1	7.06±0.08^{C}	6.75±0.12^{BC}	6.24±0.15^{B}	6.05±0.28^{Bb}	6.01±0.30^{Bb}	5.25±0.14^{Ab}	6.23±0.09^{q}
	T 2	7.06±0.08^{C}	6.75±0.12^{BC}	6.33±0.16^{B}	6.33±0.15^{Bb}	6.16±0.18^{Bb}	5.29±0.13^{Ab}	6.32±0.09^{q}
	T3	7.03±0.08^{C}	6.68±0.11^{BC}	6.29±0.14^{B}	6.29±0.19^{Bb}	6.18±0.16^{Bb}	5.13±0.18^{Ab}	6.27±0.09^{q}
	Overall means (days)	7.05±0.11^{U}	6.72±0.11^{T}	6.17±0.11^{S}	5.86±0.11^{R}	5.38±0.11^{Q}	4.16±0.11^{P}	
Flavour	Control	7.05±0.03^{D}	6.56±0.07^{C}	5.96±0.53^{Ca}	5.16±0.46^{BCa}	4.54±0.30^{Ba}	1.00±0.00^{Aa}	5.05±0.09^{p}
	T1	7.01±0.04^{D}	6.69±0.07^{C}	6.43±0.20^{Cb}	6.06±0.27^{BCb}	5.54±0.29^{Bb}	4.43±0.14^{Ab}	6.03 ±0.09^{q}
	T 2	6.95±0.05^{D}	6.66±0.08^{C}	6.54±0.21^{Cb}	6.10±0.16^{BCb}	5.62±0.27^{Bb}	4.81±0.09^{Ab}	6.11±0.09^{q}
	T3	7.03±0.06^{D}	6.73±0.11^{C}	6.33±0.12^{Cb}	6.23±0.24^{BCb}	5.72±0.23^{Bb}	4.70±0.13^{Ab}	6.12±0.09^{q}
	Overall means (days)	7.01±0.11^{U}	6.66±0.11^{T}	6.32±0.11^{S}	5.89±0.11^{R}	5.36±0.11^{Q}	3.74±10.11^{P}	
Juiciness	Control	6.95±0.06^{D}	6.69±0.16^{C}	5.74±0.61^{BCa}	5.34±0.31^{Ba}	5.03±0.32^{Ba}	1.00±0.00^{Aa}	5.22±0.08^{p}
	T1	7.05±0.06^{C}	6.68±0.13^{B}	6.44±0.21^{Bb}	6.24±0.22^{Bb}	6.08±0.21^{ABb}	5.63±0.16^{Ab}	6.30±0.08^{q}
	T 2	6.96±0.08^{C}	6.60±0.15^{BC}	6.55±0.19^{BCb}	6.39±0.16^{Bb}	6.26±0.16^{Bb}	5.83±0.13^{Ab}	6.43±0.08^{q}
	T3	7.04±0.07^{C}	6.61±0.15^{B}	6.58±0.15^{Bb}	6.39±0.16^{Bb}	6.21±0.15^{ABb}	5.89±0.07^{Ab}	6.45±0.08^{q}
	Overall means (days)	7.00±0.10^{S}	6.64±0.10^{R}	6.33±0.10^{QR}	6.24±0.10^{QR}	5.89±0.10^{Q}	4.58±0.10^{P}	
	Control	6.29±0.09^{D}	6.29±0.19^{CD}	5.91±0.46^{BC}	5.45±0.17^{Ba}	5.09±0.12^{Ba}	1.00±0.00^{Aa}	5.00±0.07^{p}
	T1	6.75±0.15^{D}	6.33±0.17^{CD}	6.23±0.23^{C}	6.03±0.02^{BCb}	5.75±0.16^{ABb}	5.56±0.06^{Ab}	6.10±0.07^{q}
	T 2	6.68±0.12^{C}	6.32±0.17^{BC}	6.30±0.21^{BC}	6.18±0.05^{ABb}	6.00±0.00^{ABb}	5.83±0.19^{Ac}	6.22±0.07^{q}
	T3	6.46±0.12^{B}	6.33±0.17^{B}	6.29±0.19^{B}	6.13±0.07^{ABb}	6.13±0.08^{ABb}	5.83±0.09^{Ac}	6.19±0.07^{q}
	Overall means (days)	6.54±0.08^{S}	6.31±0.08^{R}	6.18±0.08^{R}	5.94±0.08^{Q}	5.74±0.08^{Q}	4.55±0.08^{P}	
Overall acceptability	Control	6.54±0.18^{D}	6.48±0.13^{D}	5.55±0.56^{Ca}	5.50±0.38^{Ca}	4.59±0.03^{Ba}	1.00±0.00^{Aa}	4.94±0.08^{p}
	T1	6.68±0.12^{B}	6.68±0.15^{B}	6.48±0.20^{Bb}	6.33±0.13^{Bb}	5.73±0.18^{Ab}	5.55±0.02^{Ab}	6.24±0.08^{q}
	T 2	6.59±0.11^{B}	6.58±0.17^{B}	6.48±0.20^{Bb}	6.48±0.08^{Bb}	6.23±0.07^{Bc}	5.83±0.08^{Ac}	6.36±0.08^{q}
	T3	6.61±0.12^{C}	6.60±0.16^{C}	6.35±0.12^{Cb}	6.25±0.16^{BCb}	5.86±0.11^{ABb}	5.63±0.16^{Abc}	6.22±0.08^{q}
	Overall means (days)	6.60±0.09^{S}	6.58±0.09^{S}	6.21±0.09^{R}	6.14±0.09^{R}	5.60±0.10^{Q}	4.50±0.09^{P}	

n=5. Means with different uppercase superscripts in the same row and lowercase superscripts in the same columns are significantly different (P<0.05). Control, no irradiation and no antioxidant; T1, irradiation without antioxidant; T2, irradiation with BHA (0.02%); T3, irradiation with CLE (450 ppm equivalent phenolics).

in total plate counts, psychrotropic counts and *lactobacilli* counts in both control and treatment groups as storage period increased. A gradual increase in total plate count of seasoned ground beef product (meat ball) during refrigerated storage period was also reported by Aylin *et al. (*2010). Higher rates of increase in bacterial count were observed in non-irradiated samples. The total plate counts of control samples were reached the spoilage level of above 7 log value on the 21st day of storage. Similarly, Badr (2004) also noticed a significant increase (P < 0.05) in psychrotropic counts of all samples during storage, with higher rates of increase in non-irradiated samples. The mean psychrotropic count (log cfu/gm) values on day 14 of control samples were log 5.7, whereas these values were reached only on day 35 in treatment groups. The ability to grow rapidly under the anaerobic conditions at low temperatures and tolerance to CO_2 explain the normal dominance of *Lactic acid* bacteria in vacuum packed foods (Gonzalez *et al*. 2002). There was no significant difference (P>0.05) in total plate count and psychotropic count (log cfu/gm) among the irradiated treatment groups. This could be

due to lack of potent antimicrobial activities of BHA and plant extract.

Escherichia coli and *Salmonella* were not detected in all the treatment groups. Indicating that a dose of 3 kGy is effective in keeping the count below the detection level. Similar results were also obtained by Naik *et al.* (1994) and Sweetie *et al.* (2005) who also did not detect *Escherichia coli* in irradiated meat samples. This could be due to the high sensitivity of these organisms to irradiation.

Sensory evaluation: No significant (P>0.05) difference was noticed in the sensory attributes among the control and treatment groups upto 7 days of storage (Table 2), indicating that the gamma irradiation at doses used in the present study (3kGy) had no significant effect (P>0.05) on the initial sensory attributes of the emulsion samples. It is expected that irradiated meats, which are rich in protein and fat develops off-odour due to the radiolytic products of proteins as well as lipid oxidation by products such as mercaptomethane and dimethyl sulphide, etc. (Eirini *et al.* 2008). However, these changes are dose dependent and lower doses are usually least deteriorative (Sweetie *et al.* 2005). The results are in accordance with Javanmard *et al.* (2006), who reported that the gamma irradiation of chicken meat at the doses upto 5 kGy had no significant effects on the sensory attributes. Kim *et al.* (2002), also reported that most of the trained panelists could not differentiate the irradiated (3kGy) turkey, pork and beef from non-irradiated samples.

Upon storage period, there was a linear and highly significant (P<0.05) decrease in all the sensory attributes in the both control and treatment groups. This could be due to the gradual increase in microbial population and enzymatic degradation of nutrients (Singh *et al*. 2011). However, the deteriorative changes were faster in control samples. The mean appearance and colour values were 4.76 (between fair and slightly poor) on day 21 in control samples, whereas the values were remained around 6 (good) even on day 28 in treatment groups. Similarly, the flavor values were remained around 6 (acceptable) even on day 35 in treatment groups. Further, on day 35, BHA incorporated emulsion sample (T2) had higher flavor score. This could be due to effect of antioxidant in retarding the oxidative rancidity, off flavor associated with irradiated meat samples. The results also correlate well with TBARS values.

CONCLUSIONS

The present study showed that gamma irradiation (3kGy) significantly reduced the microbial counts of the chicken emulsion during storage. However, there was a significant rise in TBARS values of irradiated samples, indicating enhanced lipid oxidation. The inclusion of curry leaves (*Murraya koenigii*) extract at a level of 450 mg equivalent phenolics/100 gm meat inhibited ($P<0.05$) lipid oxidation in irradiated chicken meat emulsion to a much greater extent compared to control during refrigerated storage. However, synthetic antioxidant BHA had the best inhibitory effect on lipid oxidation. The sensory attributes of chicken emulsion was unaffected by irradiation at initial storage days, indicates that the gamma irradiation at doses used in the present study (3kGy) had no significant effect (P>0.05) on the sensory attributes of the emulsion samples. Thus, the present study indicated the promising potential of irradiation as an efficient preservation method for meat products and phytochemicals extracted from natural herbs like curry leaves may be used as a potential source of antioxidants to protect against oxidative rancidity without any adverse effects on sensory attributes. Meat processor can very well adopt the radiation technology along with locally available cost effective natural herbs to extend the marketable life of meat products.

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