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Combined effect of irradiation and butylated hydroxyanisole on shelf life and quality of beef at ambient temperature

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Abstract

The study was aimed to evaluate the effect of gamma irradiation and butylated hydroxyanisole (BHA) on meat quality traits and safety of beef. Samples were divided into four treatment groups treated with 0.01% BHA and gamma ray having T0 (Control), T1 (3 kGy), T2 (5 kGy) and T3 (7 kGy), respectively. The analyses were conducted at 0, 3, 5 and 7 days of interval. The study was conducted in completely randomized factorial (CRD) design. Traits evaluated were color value (L*, a*, b*), pH, drip loss, cooking loss, ERV and WHC, DM, moisture, CP, fat, ash, thiobarbituric acid reactive substances (TBARS), peroxide value (POV), free fatty acid (FFA), total coliform count (TCC), total viable count (TVC) and total yeast mould count (TYMC). Color, flavor, tenderness, juiciness and overall acceptability were significantly differed ($p<0.01$) for days of intervals. Positive and significant interaction ($p<0.01$) between treatment and days of interval was found for color value (L*, a*, b*), drip loss, cooking loss, ERV, WHC except raw pH. There were found positive and significant interaction ($p<0.01$) between treatment and days of interval for DM, CP, FFA and TBARS except POV. The TYMC and staphylococcus were significantly decreased ($p<0.05$) at different treatment groups compared to control group. Salmonella non-significantly ($p>0.05$) increased and staphylococcus significantly ($p<0.01$) decreased with increasing irradiated doses in beef. Hence, it may be concluded that BHA treated irradiated beef had a potential effect of shelf life to maintain quality and safety aspect of beef.

Introduction

Meat protein contains all essential amino acids, and vitamins, such as vitamin B12 and minerals which is benefits for human health. Meat proteins also greatly contribute to food characteristics by imparting specific functionalities, such as appearance, texture, mouth feel and satiety (Baset et al., 2003; Fiorentini et al., 2020). Due to highly biological value, perishable, nutritious, protein rich food and has a short shelf-life, it is an important factor to follow different kinds of preservation technique (Akter et al., 2009; Akhter et al., 2009; Hashem et al., 2021; Sarker et al., 2021). Although meat consumption is continuously increasing around the world but the significant portion of meat and meat products are spoiled every year. This loss is due to post harvest processing factors and microbial spoilage (Dave and Ghaly, 2011). Irradiation is a process of exposing foods to very high-energy electrons, which are similar to light waves or microwaves. This process is referred to as ionizing radiation, electron beam pasteurization, or e-beam sterilization. The radiation energy causes changes in molecules by breaking chemical bonds. At small doses, irradiation inhibits or modifies food spoilage problems, such as sprouting and ripening. Medium doses will kill or genetically alter microorganisms so they can't reproduce, which means they can no longer cause spoilage or human illness. High doses will sterilize foods and are commonly used to decontaminate herbs and spices. Gamma rays are produced by radioactive substances (Cobalt-60 or Cesium-137) that continuously emit high-energy gamma rays.

Food irradiation technique has already been applied for many decades and has been approved in 60 countries around the world (Maherani et al., 2016). The food expose to electromagnetic energy gamma ray radiation (γ-ray) can improve the microbial safety and extend its shelf life resulting in the sterility of food products. Consumption of radiated food is increasing dramatically day by day. The vast majority of consumers will readily buy irradiated food. Market access is the driving force behind the rapid growth in phytosanitary irradiation while food safety continues to an incentive for beef, poultry, and fish providers. In various countries retailers have become more open to adding irradiated foods to their shelves and the volume of irradiated items has increased dramatically, especially in the United States, New Zealand, Australia, and several Asian countries. Irradiation holds tremendous promise to increase meat safety, extend the shelf life of meat and eliminate food borne pathogens. Depending on the product characteristics and dosages applied 99 to 99.99% pathogen reductions in food through irradiation. International health and safety authorities namely Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA) and World Health Organization (WHO) reported that, medium doses up to 10 kGy of radiation can

control and destroy pathogenic and spoilage bacteria like Escherichia coli, Salmonella, Campylobacter and Listeria in food products. These are considered safe for human consumption as well as a good manufacturing practice (GMP) in food production plants (Maghraby, 2007; Gumus et al., 2008; Ayari et al., 2016). Many organizations viz. the United States Food and Drug Administration (FDA), United States Department of Agriculture (USDA), World Health Organization (WHO) and Food and Agriculture Organization (FAO) have conducted in depth safety assessments of the technology and have concluded that food irradiation is safe (Yaohua et al., 2016). The top benefits of BHA treated irradiated beef were related to making meat safer inactivating bacteria and pathogens increasing confidence in meat safety and reducing the risk of contamination and food borne diseases.

Due to oxidation of fats enhanced rancidity and heme pigments is caused by a biochemical reaction between fats and oxygen resulting in off-odors, off-flavors, discoloration. A large part of the economic loss occurred due to spoiled meat. The oxidation can be minimized by using different kind of antioxidant which may be obtained from either natural sources or synthetic forms like BHA.

Many authors studied the effect of irradiation in different days of interval with BHA and natural antioxidant on shelf life and quality of different types of meat like chicken meat (Islam et al., 2019; Disha et al., 2020), broiler meat (Rima et al., 2019), beef meatball (Islam et al., 2018; Jahan et al., 2018; Bithi et al., 2020), beef (Haque et al., 2017) and mutton (Islam et al., 2021). Only a sporadic research was found on irradiation of various meats. More study needs to find out the in-depth effect of irradiation in ambient temperature with BHA and natural antioxidant of beef to meet a challenge for meat industries in Bangladesh context. The meat industry is the largest growing business among other industries. So, this work will be treated as a new unveiled for commercial benefits to export irradiated meat in the world and will be opened a new door for the meat industries. This study is a step forward in the search for the development of nutritious, safe and healthy meat foods. Hence, the study was conducted to compare the effects of irradiation with synthetic antioxidant BHA on the quality and safety of beef at different days of interval in ambient temperature.

Materials and Methods

Sample preparation and irradiation

Fresh beef without bone was collected from different indigenous cattle from local market for experiment. After that visible fats trimmed out, meat samples were sliced into a thick piece, which is enough to be irradiated over the whole sample. Then the samples were treated with synthetic antioxidant butylated hydroxyanisole (BHA) at the rate of 0.01 %. The meat samples (500g) were transferred into zip locked bags. Each bag was considered as replicate. Samples were exposed to gamma ray at specific doses of control (non-irradiated), 3, 5, and 7 kGy in a ^{60}Co package irradiator (Gamma radiation source- Gamma Chamber -5000 (GC-5000) Models, Activity-10,473 ci (on 13.09.2012) at Bangladesh Institute of Nuclear Agriculture (BINA). The irradiation was performed at room temperature (15–20 °C). After irradiation, then the zip locked meat sample bags were kept at the condition of ambient for the analysis of different traits like sensory, proximate, biochemical and microbial analyses were performed on controls and treated samples. The analyses were conducted at different days of interval 0, 3, 5 and 7 days in ambient temperature.

Sensory Evaluation

Samples of each group were evaluated by the four honorable judges. Panelists were selected among highly trained personnel for panel test. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Saba et al., 2018; Siddiqua et al., 2018). All samples were served in the petri dishes.

Surface Color Measurements

Instrumental color measurement was carried out on meat from longissimus muscle. Color was measured at 24 h post-slaughter using Konica Minolta Chroma Meter (CR 410, Konica Minolta Sensing, Inc., Osaka, Japan), a Miniscan Spectro colorimeter programmed with the CIE Lab, (International Commission on Illumination) L*, a*, and b* system, where L* represents lightness, a* redness and b* yellowness (CIELAB, 2014). The analysis was carried out on the medial surface (bone side) of the meat at 24 h post-mortem (Rahman et al., 2020).

Physicochemical traits

Raw pH value

The pH value was measured by Hanna meat pH meter (Model HI 99163, Hanna Instruments). The pH meter was adjusted with pH 7.01 buffer solution before the measurement. The electrodes were rinsed with cleaning solution after use.

Drip loss (DL) measurement

Drip loss was measured following the procedure of Rahman et al. (2020). For DL measurement approximately 30 g sample was hung with a wire and kept in an air tight plastic container for 24 h. After 24 h the sample was weighed and calculated the difference. It was expressed as percentage.

$$\text{DL}\ (\%) = \frac{(\text{Weight of sample} - \text{weight after 24 hours chilling})}{\text{Weight of sample}} \times 100$$

Cooking loss (CL) measurement

The 30 g lamb meat sample was taken in a poly bag and heated it in water bath until the temperature rises to 71° C in sample. The beef sample with 71° C was taken out from the water bath and soaked it with tissue paper. Weight loss of the sample was measured during cooking lamb meat. The CL was calculated using following formula:

$$\text{CL}\ (\%) = \frac{(\text{Weight before cooking of sample} - \text{weight after cooking})}{\text{Weight before cooking of sample}} \times 100$$

Water holding capacity (WHC)

The WHC was measured according to the methodology of Choi et al. (2018). Thawed samples (1 g each) were wrapped in absorbent cotton and placed in a 1.5 ml centrifuge tube. The tubes with samples were centrifuged in a centrifuge separator (H1650-W Tabletop high speed micro centrifuge) at 10,000 RPM for 10 min at 4º C temperature, following which the samples were weighed. The WHC% of the sample is expressed as the following formula:

$$\text{WHC (\%)} = \frac{(\text{Weight of sample after centrifugation})}{(\text{Weight of sample before centrifugation})} \times 100$$

Extract release volume (ERV)

Blend 15g of meat for two minutes with 60 ml of extraction reagent in a blender. Extraction reagent with a pH 5.8 is prepared by taking 50 ml of 0.2 M Potassium di hydrogen phosphate and 3.72 ml of 0.2 M Sodium hydroxide and the volume made up to 200 ml with distilled water. The blended contents are quantitatively transferred to a glass funnel provided with filter paper (Whatman No.1, 18.5 cm diameter). The filter paper is folded thrice so as to make 8 sectors and filtrate collected in 100 ml measuring cylinder and the volume of filtrate collected in 15 minutes at a temperature of 20^{o} C is reported as ml of extract release volume of the meat sample.

Proximate components

Proximate components like dry matter, protein, fat, ash and moisture were determined according to AOAC (2005).

Biochemical analysis

There were three types of biochemical analysis namely Free Fatty Acid (FFA), Peroxide Value (POV) and Thiobarbituric Acid value (TBARS). Three types of analysis are discussed below.

Free Fatty Acid (%) Analysis

The Free fatty acid value was determined according to Rukunudin et al. (1998) and expressed as a percentage. The determination of FFA using following equation,

FFA (%) = ml titration × Normality of KOH × 28.2/g of sample

Peroxide Value (POV) Analysis (meq/kg)

Peroxide value (POV) was determined according to Sallam et al. (2004). The determination of peroxide value was determined using following equation:

POV was calculated and expressed as milliequivalent peroxide per kilogram of sample:

POV (meq/kg) = × 1000

Where S is the volume of titration (ml), N the normality of sodium thiosulfate solution (n = 0.01) and W the sample weight (g).

Thiobarbituric Acid Values (TBARS)

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method described by Schmedes and Homer (1989). The TBA value was expressed as milligrams malonaldehyde per kilogram of meat sample.

Microbiological Assessment

For microbial assessment total viable count (TVC), total coliform count (TCC) and total yeast-mould count (TYMC) were undertaken. A quantity of 10 g of beef meat sample was aseptically excised from stored stock sample. Each of the stored beef meat samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluents (0.1% peptone water) as per the recommendation of International Organization for Standardization (ISO, 1995). A quantity of ten (10) grams of the minced meat sample was taken aseptically transferred into a sterile container containing 90 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilutions of the samples were obtained. Later on using whirly mixture machine different serial dilutions ranging from 10-2 to 10-6 were prepared according the instruction of the standard method (ISO, 1995).

Enumeration of total salmonella spp.

Xylose Lysine Deoxycholate (XLD) agar was used which is a selective growth medium for the isolation of *Salmonella spp*. Suspend 56.68 grams in 1000 ml distilled water. Without autoclave heat with frequent agitation until the medium boils then transfer immediately to a water bath at 50°C. After cooling, pour into sterile petri dishes. For the determination of total salmonella spp. counts 0.1 ml of each ten-fold dilution was transferred and spread on triplicate XLD agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 25°C for 48–72 h. After incubation, resulting bacterial colonies was found colorless with black centers. Then 30–300 colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the count. The results of the salmonella spp. count were expressed as the number of organism of colony forming units per gram (CFU/g) of sample.

Enumeration of total staphylococcus spp.

Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus*. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. Suspend 111 grams of Mannitol Salt Agar in 1000 ml of distilled water. Then it was boiled to dissolve the medium completely. Then, the samples were sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Then the determination of total *Staphylococcus spp*. counts 0.1 ml of each ten-fold dilution was transferred and spread on triplicate MSA agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader

was used for each plate. The plates were then kept in an incubator at 25°C for 48–72 h. and 30–300 colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the count. The results of the *Staphylococcus spp*. count were expressed as the number of organism of colony forming units per gram (CFU/g) of sample.

Statistical Analysis

Data was analyzed in (4×4) factorial experiment in CRD by using SAS software. DMRT test was used to determine the significance of differences among treatments means at values ($p<0.05$).

Results and discussion

Sensory evaluation

The score range for color, flavor, tenderness, juiciness and overall acceptability at different treatments were 2.66 to 3.00, 2.75 to 3.00, 2.66 to 3.08, 2.75 to 3.00, 2.83 to 2.91, respectively and days of interval for color, flavor, tenderness, juiciness and overall acceptability were 1.49 to 4.58, 1.24 to 4.83, 1.25 to 4.83, 1.33 to 4.50, 1.41 to 4.66, respectively (Table 1). The Color was non-significantly ($p>0.05$) decreased in different treatments except T_3 compared to control group. The color value was decreased significantly with storage time which supported to the findings of Haque et al. (2017). In case of days of intervals sensory evaluation had significant ($p<0.01$) effect on color, flavor, tenderness, juiciness and overall acceptability but interaction between treatment and days of interval had no any significant ($p>0.05$) effect which was in close agreement with the findings of Habiba et al. (2021) and Rima et al. (2019).

Table 1. Effect of irradiation with BHA on sensory parameters in beef at ambient temperature

Parameters	Storage time (days)	Dose (kGy)				Mean	Level of significance		
		T_0	**T_1**	**T_2**	**T_3**		**Treat.**	**DI**	**T*DI**
Color	0	4.66±0.33	4.66±0.33	4.33±0.66	4.66±0.33	4.58^{a}±0.41	p>0.65	p<0.01	p>0.99
	3	3.33±0.33	3.33±0.33	2.66±0.33	3.33±0.33	3.16^{b}±0.33			
	5	2.33±0.33	2.33±0.33	2.33±0.33	2.33±0.33	2.33^{c}±0.33			
	7	1.66±0.66	1.33±0.33	1.33±0.33	1.66±0.66	1.49^{d} ±0.49			
	Mean	3.00^{a}±0.41	2.91^{a}±0.33	2.66^{a}±0.41	2.91^{a}±0.41				
Flavor	0	4.66±0.33	5.00±0.00	4.66±0.33	5.00±0.00	4.83^{a}±0.16	p>0.37	p<0.01	p>0.80
	3	3.33±0.33	3.00±0.00	3.33±0.33	3.33±0.33	3.25^{b}±0.24			
	5	2.00±0.00	2.66±0.33	2.33±0.33	2.33±0.33	2.33^{c}±0.24			
	7	1.00±0.00	1.33±0.33	1.33±0.33	1.33±0.33	1.24^{d} ±0.24			
	Mean	2.75^{a}±0.16	3.00^{a}±0.16	2.75^{a}±0.33	2.91^{a}±0.24				
Juiciness	0	4.66±0.33	5.00±0.00	4.66±0.33	5.00±0.00	4.83^{a}±0.16	p>0.01	p<0.01	p>0.94
	3	3.00±0.00	3.33±0.33	3.33±0.33	3.33±0.33	3.25^{b}±0.24			
	5	2.00±0.00	2.66±0.33	2.33±0.33	2.00±0.00	2.25^{c}±0.16			
	7	1.00±0.00	1.33±0.33	1.33±0.33	1.33±0.33	1.25^{d} ±0.24			
	Mean	2.66^{b}±0.08	3.08^{a}±0.24	2.91^{ba}±0.33	2.91^{ba}±0.16				
Tenderness	0	4.66±0.33	4.33±0.33	4.33±0.66	4.66±0.33	4.50^{a}±0.41	p>0.77	p<0.01	p>0.97
	3	3.33±0.33	3.33±0.33	3.33±0.33	3.33±0.33	3.33^{b}±0.33			
	5	2.00±0.00	2.33±0.33	2.33±0.33	2.33±0.33	2.25^{c}±0.24			
	7	1.33±0.33	1.00±0.00	1.33±0.33	1.66±0.33	1.33^{d} ±0.24			
	Mean	2.83^{a}±0.24	2.75^{a}±0.24	2.83^{a}±0.41	3.00^{a}±0.33				
Overall acceptability	0	4.66±0.33	4.66±0.33	4.66±0.33	4.66±0.33	4.66^{a}±0.33	p>0.97	p<0.01	p>0.99
	3	3.33±0.33	3.00±0.00	3.33±0.33	3.00±0.00	3.16^{b}±0.16			
	5	2.33±0.33	2.33±0.33	2.33±0.33	2.33±0.33	2.33^{c}±0.33			
	7	1.33±0.33	1.33±0.33	1.33±0.33	1.66±0.33	1.41^{d} ±0.33			
	Mean	2.91^{a}±0.33	2.83^{a}±0.24	2.91^{a}±0.33	2.91^{a}±0.24				

Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. Different superscripts in different treatments groups and days of interval differ significantly ($p<0.05$). T_0 (Control group), T_1(3 kGy), T_2(5 kGy), T_3(7 kGy), DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Intervals

Instrumental color values

The average values at treatments groups and different days of interval for L*, a* and b* was 39.91 to 42.62, 11.46 to 12.08, 11.83 to 14.00 and 37.82 to 46.02, 10.35 to 12.61, 9.02 to15.95, respectively (Table 2). The L* value of meat sample was significantly ($p<0.01$) increased in treated groups except T_1 compared to control. Higher L* value (42.62) was observed in treated T_2 (5 kGy) treatment. The results shown that irradiated treated meat for a* value was lower as compared to control samples. The results a* scale agreed with the findings of Liming et al. (2017) who observed a* of spicy beef jerky was significantly ($p<0.05$) decreased as the irradiation energy increased to 8 kGy. Their results were supported to the present study with decreasing a* value due to treated of BHA. The b* value of the irradiated sample was increased gradually and had a significant ($p<0.01$) effect with the increase of irradiation doses and found higher value of b* (14.00) in treated T_3 (7 kGy). The results of present study (CIE lab L*a*b* scale) agreed with the findings of Muhammad et al. (2019).

Table 2. Effect of irradiation with BHA on instrumental color valu in beef at ambient temperature

Parameters		Storage time (days)	Dose (kGy)				Mean	Level of significance		
			T_0	T_1	T_2	T_3		Treat.	DI	T*DI
Color	L*	0	36.42±0.85	34.85±0.52	40.60±0.58	39.42±1.31	37.82^{d}±0.81	p<0.01	p<0.01	p<0.01
		3	41.36±0.72	40.12±0.31	42.64±0.55	41.88±0.01	41.5^{b}±0.39			
		5	38.85±0.14	38.84±0.33	41.13±0.51	40.45±0.26	39.82^{c}±0.31			
		7	45.45±0.49	45.84±0.28	46.12±0.42	46.66±0.26	46.02^{a}±0.36			
		Mean	40.52^{b}±0.55	39.91^{b}±0.36	42.62^{a}±0.51	42.10^{a}±0.46				
	a*	0	13.30±0.21	13.21±0.05	12.05±0.02	11.87±0.05	12.61^{a}±0.07	p>0.11	p<0.01	p<0.01
		3	12.22±0.54	11.33±0.47	12.02±0.41	11.67±0.18	11.81^{b}±0.4			
		5	12.84±0.21	12.33±0.06	13.07±0.07	12.52±0.03	11.81^{b}±0.08			
		7	9.96±0.07	11.39±0.13	10.28±0.06	9.76±0.10	10.35^{c}±0.09			
		Mean	12.08±0.34	12.07±0.17	11.86±0.14	11.46±0.83				
	b*	0	7.66±0.38	9.43±0.21	9.57±0.11	9.44±0.15	9.02^{d}±0.21	p<0.01	p<0.01	p<0.01
		3	12.00±0.22	11.19±0.41	10.65±0.19	14.52±0.26	12.09^{c}±0.27			
		5	11.21±0.05	14.29±0.06	17.19±0.12	16.30±0.22	14.75^{b}±0.11			
		7	16.43±0.34	15.80±0.20	15.84±0.04	15.74±0.28	15.95^{a}±0.21			
		Mean	11.83^{d}±0.24	12.68^{c}±0.22	13.31^{b}±0.11	14.00^{a}±0.23				

Different superscripts in same row of different treatment groups and days of interval differ significantly (p<0.05), T_0= Control group, T_1= 3 kGy gamma ray, T_2= 5 kGy gamma ray, T_3= 7 kGy gamma ray, DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Intervals.

Physico-chemical properties

The average values of treatments groups for pH (6.35 to 6.53), drip loss (3.15 to 3.54), cooking loss (34.34 to 36.69), extract release value (22.00 to 33.33), and WHC (85.07 to 86.29) were shown in Table 3. The conversion of muscle to meat is the key point of muscle pH. After slaughter the animals at early post-mortem stage muscle pH remains around 7.0–7.2 and converted to meat pH 5.5–5.9 (Rahman et al. 2020). The result shown that raw pH values slightly decreased in all treatment groups but increased significantly (p<0.01) in all groups with the increasing storage time and found higher value at 7th day (7.56) resulting increase of free fatty acids due to rancidity. Haque et al. (2017) found same trend in beef irradiation. Similar results were obtained by Al-Bachir et al. (2010) for the influence of gamma irradiation of chicken kebab. Similarly, previous studies reported that a change in pH of irradiated meat products had no impact on their quality attributes (Badr and Mahmoud, 2011; Ham et al., 2017). Bacteria and mold have a tendency to increase with increasing storage time that affects the increasing ultimate pH. Similar results was found by Biswas et al. (2004) during storage time using antioxidant BHA and BHT in precooked pork patties. Physicochemical traits as DL, CL, WHC, ERV had significant (p<0.01) effect on treatments, days of interval and interaction between treatments and days of interval. A combination of low pH and high temperature can promote the proteins denaturation that leads to greater drip loss and make the meat pale. The results showed that cooking loss was significantly increased with irradiation doses. Irradiation as well as storage time decreases muscle fiber that was the cause of increased cooking losses. This result was in close agreement with the findings of Yoon (2003) who reported that irradiated chicken breast meat had more cooking loss than non-irradiated meat. ERV value estimates the relations of microbial load. The result shown that among treatment groups water volume gradually decreased that is ERV gradually decreased to indicate that microbial load increased and also ERV decreased with increasing storage period at 7th days (13.91). The WHC decreased with increasing storage period at 7th day (81.56). The increasing storage period caused proteolysis and occurred off flavor to gear up spoilage. The ERV and WHC is also key indicator of spoilage of meat samples. The result showed significant (p<0.01) effect on treatments, and interaction between treatment and days of interval. These results were agreed with Osburn and Keeton (2004) who reported that the increase in moisture content can be attributed to the large water holding capacity of the hydrocolloid gel.

Table 3. Effect of irradiation with BHA on physicochemical properties in beef at ambient temperature

Parameters	Storage time (days)	Dose (kGy)				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
Raw pH	0	5.77 ±0.03	5.77±0.08	5.83±0.06	5.70±0.05	5.76^{c}±0.05	p>0.10	p<0.01	p>0.65
	3	6.30±0.05	6.30±0.05	6.33±0.08	6.26±0.08	6.30^{b}±0.06			
	5	6.33±0.08	6.06±0.33	6.33±0.08	6.30±0.11	6.25^{b}±0.15			
	7	7.67±0.08	7.30±0.05	7.63±0.08	7.56±0.08	7.56^{a}±0.07			
	Mean	6.52±0.06	6.35±0.08	6.53±0.07	6.45±0.08				
Drip loss	0	2.99±0.04	1.02 ±0.00	1.04±0.02	1.04±0.02	1.52^{d}±0.02	p<0.01	p<0.01	p<0.01
	3	3.21±0.05	3.46±0.02	3.10±0.01	3.75±0.09	3.38^{c}±0.04			
	5	3.71±0.09	4.03±0.09	3.82±0.04	3.84±0.07	3.85^{b}±0.05			
	7	4.24±0.08	4.35±0.08	4.65±0.04	4.39±0.07	4.41^{a}±0.06			
	Mean	3.54^{a}±0.06	3.22^{cb}±0.04	3.15^{c}±0.03	3.25^{b}±0.06				
Cooking loss (%)	0	29.55±0.72	28.34±0.29	28.07±0.08	27.44±0.09	28.35^{d}±0.29	p<0.01	p<0.01	p<0.01
	3	29.80±0.22	31.34±0.38	33.99±0.12	38.22±0.45	33.34^{c}±0.29			
	5	37.55±0.28	41.63±0.52	40.38±0.78	40.29±0.28	39.96^{b}±0.46			
	7	40.45±0.46	39.50±0.48	41.83±0.19	40.46±0.29	40.57^{a}±0.35			
	Mean	34.34^{c}±0.42	35.20^{b}±0.41	36.06^{a}±0.29	36.69^{a}±0.27				
Extract release value	0	33.33±0.33	33.66±0.33	34.66±0.57	34.66±0.33	34.08^{a}±0.57	p<0.01	p<0.01	p<0.01
	3	28.00±0.57	27.00±0.57	26.33±0.33	25.00±0.57	26.58^{b}±0.51			
	5	17.00±0.57	15.66±0.33	16.33±0.36	16.00±0.57	16.25^{c}±0.45			
	7	15.00±0.33	15.00±0.57	13.66±0.57	12.33±0.33	13.91^{d}±0.32			
	Mean	23.33^{a}±0.45	22.33^{a}±0.64	22.49^{a}±0.87	22.00^{b}±0.57				
Water holding capacity	0	89.02±0.29	88.81±0.16	88.47±0.17	88.13±0.26	88.61^{c}±0.22	p<0.01	p<0.01	p>0.89
	3	87.47±0.37	87.22±0.17	87.66±0.67	86.92±1.08	87.31^{c}±0.42			
	5	86.11±0.11	85.72±0.41	85.52±0.55	84.68±0.63	85.51^{c}±0.24			
	7	82.59±0.33	81.89±0.58	81.19±0.57	80.57±0.53	81.56^{a}±0.50			
	Mean	86.29^{a}±0.27	85.91^{a}±0.33	85.71^{ab}±0.49	85.07^{b}±0.23				

Different superscripts in same row of different treatment groups and days of interval differ significantly (p<0.05) T_0= Control group, T_1= 3kGy gamma ray, T_2= 5kGy gamma ray, T_3= 7kGy gamma ray, DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Intervals

Proximate coponents

The average value of DM, CP, EE, ash and moisture content of treatment groups were 24.55 to 25.70, 22.61 to 23.94, 1.47 to 1.77, 1.00 to1.21, 74.29 to 75.45 respectively (Table 4) and days of interval for DM, CP, EE, ash and moisture content were 23.70 -26.98, 22.59-23.84, 1.07-2.79, 0.98-1.31 and 73.02-76.30 respectively. DM, CP, EE, and moisture were found significant differed ($p<0.01$). There were found positive significant effect among the DM, CP, EE, and moisture between treatment and days of interval except ash content. Rima et al. (2019) found increased of CP and EE as per increasing level of irradiated doses. These results were similar to the present study. These findings were also in close agreement with those reported by Gecgel (2013), Suradkar et al. (2013), Al-Bachir and Zeinou (2014) and Zargar et al. (2017).

Table 4. Effect of irradiation with BHA on proximate components in beef at ambient temperature

Parameters	Storage time (days)	Dose (kGy)				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
DM (%)	0	24.55±.28	26.98±0.08	27.22±0.31	27.16±0.14	26.98^{a}±0.20	p<0.01	p<0.01	p<0.01
	3	25.72±0.27	26.48±0.32	26.84±0.27	27.03±0.15	26.52^{b}±0.25			
	5	23.39±0.18	24.34±0.12	24.18±0.09	24.17±0.04	24.02^{c}±0.10			
	7	22.56±0.28	23.24±0.19	24.55±0.25	24.46±0.27	23.70^{c}±0.24			
	Mean	24.55^{c}±0.25	25.26^{b}±0.17	25.70^{a}±0.23	25.70^{a}±0.15				
CP (%)	0	24.18±0.11	25.29±0.16	21.91±0.19	23.99±0.05	23.84^{a}±0.11	p<0.01	p<0.01	p<0.01
	3	22.67±0.18	23.97±0.08	23.96±0.02	22.89±0.07	23.37^{b}±0.7			
	5	22.03±0.02	24.08±0.50	22.77±0.46	22.77±0.46	22.91^{c}±0.36			
	7	21.57±.07	22.43±0.12	23.23±0.23	23.15±0.05	22.59^{c}±0.10			
	Mean	22.61^{c}±0.09	23.94^{a}±0.21	22.97^{b}±0.22	23.20^{b}±0.15				
EE (%)	0	2.06±0.08	3.09±0.06	3.19±0.09	2.81±0.09	2.79^{a}±0.05	p<0.01	p<0.01	p<0.01
	3	1.84±0.08	1.53±0.08	1.33±0.08	1.53±0.03	1.56^{b}±0.06			
	5	1.24±0.03	1.00±0.01	1.10±0.05	1.60±0.05	1.23^{c}±0.02			
	7	0.75±0.02	1.14±0.03	1.22±0.04	1.15±0.02	1.07^{d}±0.02			
	Mean	1.47^{b}±0.05	1.69^{a}±0.04	1.71^{a}±0.06	1.77^{a}±0.04				
Ash (%)	0	1.09±0.06	1.80±0.05	1.23±0.04	1.14±0.00	1.31^{a}±0.03	P<0.01	p<0.01	P<0.01
	3	1.01±0.00	1.03±0.00	0.99±0.00	1.04±0.03	1.02^{b}±0.0			
	5	0.94±0.02	0.96±0.00	0.97±.02	1.04±0.02	0.98^{b}±0.0			
	7	0.96±0.00	1.07±0.05	0.93±0.03	0.93±0.02	0.97^{b}±0.02			
	Mean	1.00^{b}±0.02	1.21^{a}±0.05	1.03^{b}±.02	1.04^{b}±0.01				
Moisture (%)	0	75.45±0.27	73.02±0.32	72.78±0.42	72.84±0.15	73.02^{d}±0.25	p<0.01	p<0.01	p<0.01
	3	74.28±0.18	73.52±0.12	73.16±0.26	72.97±0.04	73.48^{c}±0.14			
	5	76.61±0.28	75.66±0.19	75.82±0.25	75.83±0.27	75.98^{b}±0.18			
	7	73.44±0.28	76.76±0.08	75.45±0.31	75.54±0.14	76.30^{a}±0.16			
	Mean	75.45^{a}±0.25	74.74^{b}±0.17	74.30^{c} ±0.31	74.29^{c}±0.15				

Different superscripts in different treatment groups and days of interval differ significantly ($p<0.05$), T_0= Control group, T_1= 3kGy gamma ray, T_2= 5kGy gamma ray, T_3= 7kGy gamma ray, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Interval

Biochemical properties

The average value of FFA, POV and TBARS values in treatment groups were 4.63 to 5.49, 2.51 to 3.14 and 0.42 to 0.59 and, respectively (Table 5). The result shown that compared to the control, the inclusion of BHA retarded effectively to the oxidative process of irradiation dose level in treatment groups but with increasing storage time, values were higher than control values. Irradiation induced lipid oxidation up to 3 days of storage time after that spoilage started with extended storage time. The FFA, POV and TBARS of treatments groups were showed closed values and days of interval significantly ($p<0.01$) increased. These results did not agree to the findings of Haque et al. (2017). The FFA, TBARS and POV were found positive significant ($0<0.01$) interaction between treatments and days of interval. These findings were in close agreement with those reported by Iulia et al. (2013), Quattara et al. (2002), Haque et al. (2017) and Al-Bachir and Zeinou, (2014).

Table 5. Effect of irradiation with BHA on biochemical parameters in beef at ambient temperature

Parameters	Storage time (days)	Dose (kGy)				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
FFA (%)	0	1.13±0.00	1.38±0.04	1.29±0.04	1.29±0.09	1.27^{d}±0.04	p<0.01	p<0.01	p<0.01
	3	1.72±0.00	1.75±0.00	1.76±0.00	1.99±0.06	1.80^{c}±0.01			
	5	6.70±0.19	6.97±0.07	7.51±0.15	7.88±0.13	7.26^{b}±0.13			
	7	8.99±0.27	9.71±0.3	9.71±0.21	10.83±0.26	9.81^{a}±0.15			
	Mean	4.63^{c}±0.11	4.95^{b}±0.03	5.07^{b}±0.1	5.49^{a}±0.13				
POV (meq/kg)	0	0.11±0.00	0.11±0.00	0.11±0.00	0.15±0.01	0.12^{c}±0.04	p<0.01	p<0.01	p<0.01
	3	0.05±0.00	0.05±0.00	0.06±0.00	0.05±0.00	0.05^{c}±0.00			
	5	1.63±0.04	1.60±0.02	1.69±0.02	1.66±0.01	1.64^{b}±0.2			
	7	2.56±0.06	2.51±0.26	2.93±0.10	3.14±0.21	2.78^{a}±0.15			
	Mean	1.09^{b}±0.04	1.07^{b}±0.20	1.19^{ab}±0.02	1.25^{a}±0.03				
TBARS (mg-MA/kg)	0	0.09±0.00	0.13±0.02	0.12±0.00	0.12±0.00	0.11^{d}±0.00	p<0.01	p<0.01	p<0.01
	3	0.22±0.00	0.28±0.00	0.25±0.02	0.26±0.00	0.25^{c}±0.00			
	5	0.66±0.01	0.69±0.00	0.87±0.00	0.92±0.00	0.79^{b}±0.00			
	7	0.72±0.00	0.84±0.02	0.99±0.01	1.08±0.06	0.91^{a}±0.03			
	Mean	0.42^{d}±0.01	0.48^{c}±0.01	0.56^{b}±0.00	0.59^{a}±0.02				

Different superscripts in same row of different treatment groups and days of interval differ significantly ($p<0.05$) T_0= Control group, T_1= 3kGy gamma ray, T_2= 5kGy gamma ray, T_3= 7kGy gamma ray, DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Intervals

Microbiological assessment

The TCC, TVC, TYMC, salmonella and staphylococcus counts are shown in Table 6. The average value of treatment groups were 2.83 to 3.16, 5.21to 5.61, 2.87 to 3.15, 3.79 to 3.43, 3.67 to 4.19 (logCFU/g), respectively. The results showed that different treatments groups and different days of intervals with storage time values were increased significantly with irradiation doses of 3, 5 and 7 kGy compared to control group and significantly increased with increasing storage time under ambient temperature. Ferawati et al. (2015) observed that total microbial loads of the irradiated samples were lower than control. These findings were very close to those reported by Reddy et al. (2017), Inamura et al. (2012), and Afrin et al. (2017). These results were similar to the present study. Salmonella non-significantly increased and staphylococcus significantly ($p<0.01$) decreased with increasing irradiated doses in beef. Several studies showed that low doses (2, 4, and 6 kGy) of gamma irradiation reduced yeasts, molds, coliforms, E. coli and *Staphylococcus aureus* counts to safe levels in ground beef samples (Ayari et al., 2016; Song et al., 2016). Similar results were also found by the present study.

Table 6. Effect of irradiation with BHA on different microbe's population in beef at ambient temperature

Parameters	Storage time (days)	Dose (kGy)				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
TCC (logCFU/g)	0	1.93±0.01	2.24±0.08	2.74±0.04	2.74±0.04	2.41^{d}±0.05	p<0.01	p<0.01	p<0.01
	3	2.66±0.01	2.66±0.04	2.53±0.09	2.57±0.07	2.60^{c}±0.06			
	5	3.21±0.05	3.35±0.04	3.43±0.06	3.49±0.02	3.37^{b}±0.05			
	7	3.53±0.12	3.71±0.06	3.68±0.03	3.83±0.06	3.69^{a}±0.08			
	Mean	2.83^{c}±0.09	2.99^{b}±0.07	3.09^{a}±0.02	3.16^{a}±0.02				
TVC (logCFU/g)	0	3.65±0.02	3.56±0.03	3.52±0.04	3.52±0.03	3.56^{d}±0.08	p<0.01	p<0.01	p<0.01
	3	3.74±0.03	3.79±0.02	3.82±0.07	3.85±0.05	3.80^{c}±0.08			
	5	6.25±0.04	6.34±0.05	6.87±0.12	7.29±0.08	6.68^{b}±0.07			
	7	7.23±0.00	7.40±0.19	7.56±0.19	7.80±0.11	7.50^{a}±0.12			
	Mean	5.21^{c}±0.04	5.27^{c}±0.08	5.44^{b}±0.10	5.61^{a}±0.06				
TYMC (logCFU/g)	0	1.67±0.02	1.80±0.03	1.81±0.04	1.75±0.05	1.76^{d}±0.04	p>0.18	p<0.01	p>0.99
	3	1.92±0.01	2.01±0.06	2.07±0.08	2.16±0.10	2.04^{c}±0.05			
	5	2.79±0.17	2.92±0.31	3.06±0.14	3.18±0.10	2.99^{b}±0.18			
	7	5.12±0.45	5.15±0.17	5.32±0.07	5.50±0.24	5.27^{a}±0.15			
	Mean	2.87^{a}±0.16	2.97^{a}±0.14	3.06^{a}±0.09	3.15^{a}±0.12				
Salmonella (logCFU/g)	0	3.37±0.14	3.51±0.19	3.67±0.27	3.67±0.23	3.55^{c}±0.15	p>0.55	p<0.01	p<0.01
	3	3.21±0.05	4.17±0.02	3.93±0.01	3.76±0.04	3.77^{cb}±0.02			
	5	4.23±0.18	3.68±0.24	3.68±0.09	3.66±0.03	3.81^{b}±0.12			
	7	4.35±0.09	4.29±0.15	4.39±0.01	4.25±0.13	4.32^{a}±0.06			
	Mean	3.79^{a}±0.11	3.91^{a}±0.15	3.92^{a}±0.09	3.83^{a}±0.10				
Staphycoccus (logCFU/g)	0	3.67±0.17	3.67±0.12	3.53±0.18	3.58±0.23	3.61^{b}±0.11	p<0.01	p<0.01	p<0.01
	3	4.32±0.06	3.63±0.10	3.65±0.06	3.57±0.07	3.79^{b}±0.05			
	5	4.27±0.15	4.13±0.03	3.87±0.04	3.84±0.08	4.03^{a}±0.05			
	7	4.52±0.14	4.31±0.06	3.64±0.21	3.82±0.06	4.07^{a}±0.10			
	Mean	4.19^{a}±0.13	3.94^{b}±0.07	3.67^{c}±0.12	3.70^{c}±0.12				

Different superscripts in same row of different treatment groups and days of interval differ significantly ($p<0.05$), T_0= Control group, T_1= 3kGy gamma ray, T_2= 5kGy gamma ray, T_3= 7kGy gamma ray, DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Intervals.

Conclusion

It is revealed from the study that the irradiation dose 5 kGy performed better regarding to overall acceptability, nutritional aspect, and microorganism control and for shelf life extension of beef. Therefore, it may be concluded that BHA treated (5 kGy) beef expose to gamma ray had a potential effect of shelf life extension to maintain nutritional quality and safety aspect of beef.

Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

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