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Evaluating gamma irradiation and butylated hydroxyanisole (BHA) as preservation strategies for frozen beef

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Abstract

This study examined the effects of butylated hydroxyanisole (BHA), a synthetic antioxidant, in conjunction with gamma irradiation on the biochemical characteristics and microbiological population of beef during frozen storage at various time intervals. BHA (0.01%) was applied to fresh boneless beef samples, and a ⁶⁰Co source was used to expose them to varying doses of gamma radiation (0, 3, 5, and 7 kGy). At intervals of 0, 15, 30, and 120 days, treated samples were kept at -20°C and examined for lipid oxidation markers such as free fatty acid (FFA), peroxide value (POV), and thiobarbituric acid reactive substances (TBARS). Additionally, microbiological parameters such as total coliform count (TCC), total viable count (TVC), total yeast and mold count (TYMC), *Salmonella* spp., and *Staphylococcus* spp. were measured. FFA and TBARS were substantially higher ($p < 0.01$) in all trial groups than in the control, while POV was lower in all but T1 treatment groups. These oxidative markers also dramatically increased with the storage period. Microbial studies found that although TVC, TYMC, and *Salmonella* spp. increased in irradiated groups, they remained within acceptable ranges. *Staphylococcus* spp. levels considerably decreased ($p < 0.01$) with increasing irradiation doses. Interaction effects between irradiation dosage and storage period were substantial ($p < 0.01$) for most biochemical and microbiological parameters. The research indicates that irradiation along with BHA can enhance beef's microbial safety and oxidative stability during frozen storage if proper dosage levels and storage conditions are carefully maintained.

Introduction

Beef, obtained from cattle carcasses, is an essential red meat that significantly contributes to a balanced diet by providing higher levels of protein, vitamins, and minerals compared to plant-based foods (Sayeed et al., 2023; Azad et al., 2022). Despite its nutritional benefits, beef is highly susceptible to spoilage caused by microbial growth, enzymatic reactions, and oxidative processes, which collectively reduce its shelf life and negatively impact consumer acceptance (Chakrabarty et al., 2024; Dave & Ghaly, 2011). To mitigate these issues, irradiation has been widely explored as an effective preservation method to reduce microbial populations and extend shelf life (Rima et al., 2019; Sadakuzzaman et al., 2021). For example, applying 2 kGy irradiation to Black Bengal goat meat effectively maintained microbial safety (Ruman Khan et al., 2017). However, biochemical markers of spoilage, such as free fatty acids (FFA), peroxide value (POV), and thiobarbituric acid reactive substances (TBARS), tend to increase with higher irradiation doses and prolonged storage durations (Rima et al., 2019; Yim et al., 2019). Similarly, frozen duck meat exposed to 7 kGy irradiation exhibited elevated TBARS, POV, and total volatile basic nitrogen (TVBN) values compared to non-irradiated controls (Zhang et al., 2016). While irradiation effectively inhibits microbial growth, microbial counts—including total coliform count (TCC), total yeast and mold count (TYMC), and total viable count (TVC)—can increase during extended storage, underscoring the necessity for preservation strategies that balance microbial suppression with oxidative stability (Rima et al., 2019). In this regard, combining irradiation with synthetic antioxidants such as butylated hydroxyanisole (BHA) presents a promising approach by slowing lipid oxidation and maintaining freshness without compromising sensory or nutritional quality (Islam et al., 2025; Sadakuzzaman et al., 2024; Liu et al., 2015). Natural antioxidants have also demonstrated potential in meat preservation. *Moringa oleifera* leaf extract, for instance, has been shown to preserve the quality of beef meatballs comparably to BHA, with a 0.3% concentration providing optimal microbial stability and sensory acceptability (Islam et al., 2018). These findings suggest that both synthetic and plant-based antioxidants, particularly when used in combination with irradiation, offer effective preservation solutions. Understanding species-specific meat quality characteristics is crucial for developing tailored preservation methods.

Comparative research between Black Bengal goats and Indigenous sheep revealed that sheep meat exhibited greater tenderness and lipid oxidation, while goat meat was preferred for its distinctive flavor and higher acceptability (Akter et al., 2022; Murshed et al., 2014). Moreover, studies on organic sheep production practices highlight feeding, healthcare, and housing systems that influence meat quality and may limit the use of synthetic additives (Sarker et al., 2017).

Advances in technology have introduced non-destructive analytical tools such as Near-Infrared Spectroscopy (NIRS) coupled with Artificial Intelligence (AI) to enhance meat quality assessment. Machine learning algorithms—including Support Vector Machines (SVM), logistic regression, and neural networks—have achieved classification accuracies exceeding 84% in distinguishing cooked broiler and duck meat (Bristy et al., 2025). These techniques facilitate rapid authentication and fraud detection in the meat industry. Additionally, predictive models using filter paper wetness methods have successfully estimated drip loss and water-holding capacity, establishing essential benchmarks for broiler meat quality classification (Sarkar et al., 2024). The rigor state of meat also significantly affects quality attributes; post-rigor aging for 24 hours has been shown to improve tenderness, flavor, and color in broiler meat (Khatun et al., 2025). Food safety concerns related to antibiotic residues in meat have been rigorously investigated. One study reported that residues of tetracycline, ciprofloxacin, and enrofloxacin in various beef tissues remained below international maximum residue limits; however, it emphasized the importance of stringent veterinary drug administration and regulatory oversight to safeguard public health (Kamal et al., 2025).

The objective of this study was to investigate the effect of gamma irradiation combined with butylated hydroxyanisole on the biochemical properties and microbial population of beef stored at freezing temperatures over different time intervals.

Materials and Methods

Sample Collection and Treatment

Approximately 3 kg of fresh boneless beef was collected from indigenous cattle at a local market. The beef was marinated with 0.01% butylated hydroxyanisole (BHA) and divided into 500 g portions. Each portion was sealed in a zip-lock bag with three replications per treatment group. The samples were divided into four groups: control (no irradiation) and three treatment groups subjected to 3, 5, and 7 kGy gamma irradiation, respectively. Irradiation was carried out using a 60Co gamma chamber (Model GC-5000, Activity: 10,473 Ci) at the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, at room temperature (15–20°C). The exposure times for 3, 5, and 7 kGy doses were 54:04, 1:30:06, and 2:06:08, respectively. All irradiated and control samples were stored at –20°C and analyzed at 0, 15, 30, and 120 days of storage.

Chemicals and Reagents

Chemicals used in the study included BHA, peptone, potato dextrose agar, MacConkey agar, chloroform, sulphuric acid, sodium hydroxide, copper sulphate, starch solution, phenolphthalein, and various buffer solutions. All chemicals were of analytical grade and used as per standard protocols.

Apparatus and Equipment

A range of instruments was used, including a muffle furnace, Minolta colorimeter, refrigerator, Hanna pH meter, microscope, Kjeldahl and Soxhlet apparatus, digital shaking bath, incubator, oven, homogenizer, centrifuge, spectrophotometer, magnetic stirrer, micro-grinder, meat grinder, microwave oven with grill, digestion system, precision balances, water pump, and general laboratory tools. Equipment was thoroughly cleaned, autoclaved, and dried prior to use.

Irradiation Procedure

Post-marination, the beef samples were assigned to four irradiation treatments (0, 3, 5, and 7 kGy). Irradiation was conducted using a cobalt-60 gamma irradiator (GC-5000) at BINA. Each dose group received its corresponding radiation exposure and was then immediately frozen at –20°C for subsequent biochemical and microbiological evaluation.

Biochemical Analysis

Lipid oxidation and fat degradation were measured using three markers: peroxide value (POV), free fatty acid (FFA), and thiobarbituric acid reactive substances (TBARS). POV was determined following the method of (Sallam et al. 2004) using the formula: $POV (meq/kg) = (S \times N) / W \times 1000$, where S is the titration volume in ml, N is the normality of sodium thiosulfate (0.01N), and W is the sample weight in grams. FFA content was calculated based on (Rukunudin et al. 1998) as: $FFA (\%) = \text{titrate (ml)} \times KOH \text{ normality} \times 28.2 / \text{sample weight (g)}$. TBARS was assessed following (Schmedes and Holmer 1989), and results were expressed in mg malonaldehyde per kg of sample.

Microbiological Analysis

Microbial counts were conducted according to (Ikhlas et al. 2012). Total coliform count (TCC) was enumerated on MacConkey agar incubated at 35°C for 24–48 hours. Total viable count (TVC) was assessed using plate count agar (PCA) under similar conditions. Total yeast and mold count (TYMC) was determined using potato dextrose agar incubated at 25°C for 48–72 hours. Salmonella spp. were detected on XLD agar based on colony morphology (black-centered colonies), while Staphylococcus spp. were identified using Mannitol Salt Agar (MSA). All counts were calculated as colony-forming units per gram (CFU/g).

Statistical Analysis

The data were analyzed using a 4×4 factorial design in a completely randomized design (CRD). Statistical analysis was performed using SAS software. Differences among treatment means were tested using Duncan's Multiple Range Test (DMRT), with significance considered at the 1% level ($p < 0.01$).

Results and Discussion

Effect on biochemical properties

The impact of BHA on the biochemical properties of frozen beef such as POV, FFA and TBARS value is presented in Table 1. The average values of FFA, POV and TBARS were found 0.19, 2.33, 0.23; 0.21, 2.36, 0.27; 0.21, 2.29, 0.31 and 0.24, 2.29, 0.27, respectively in T₀, T₁, T₂ and T₃ treatment groups. The FFA and TBARS had significant (p<0.01) impact on four groups of treatment but POV showed insignificant (p>0.05) effect. Average values of FFA, POV, and TBARS were 0.12, 1.26, 0.11; 0.31, 1.78, 0.38; 0.34, 2.56, 0.46; and 0.08, 3.67, 0.11, respectively, over storage time. These numbers showed a substantial (p<0.01) effect for intervals of 0, 30, 60, and 120 days. Treatment and interval days had a significant (p<0.01) impact on the interaction for FFA, POV, and TBARS levels. (Gecgel, 2013) showed that radiation significantly (p<0.05) affected FFA values. (Arshad et al. 2020) found that when irradiation doses rose, the FFA% of frozen duck flesh dropped, but the current study did not support their findings. (Quattara et al., 2002) discovered the POV value was raised in beef with the increasing of irradiation doses which contrast with our results. According to the current investigation, POV was improved during the term of storage in comparison to the group under control, which was supported by the findings of (Biplob et al., 2024; Shohiduzjaman et al., 2024; Javanmard et al., 2006). Increased TBA level in cooked or processed beef with a 4.5 kGy gamma radiation exposure was reported by some sources (Rady et al., 2002). This outcome was identical with the findings of present study. The findings of other researchers about TBARS levels in various meats and meat-based products have increased due to exposure to radiation doses and storage periods (Masum et al., 2025; Arshad et al., 2020; Badr, 2004; Kanatt et al., 2006; Chen et al., 2007).

Table 1. Effect on biochemical properties of beef

Parameters	Storage time(days)	Dose (kGy)				Mean	Level of significance		
		T ₀	T ₁	T ₂	T ₃		Treat.	DI	T*DI
FFA (%)	0	0.11±0.00	0.11±0.00	0.11±0.00	0.15±0.01	0.12 ^b ±0.00	p<0.01	p<0.01	p<0.01
	30	0.22±0.03	0.28±0.03	0.35±0.04	0.37±0.03	0.31 ^a ±0.03			
	60	0.36±0.04	0.33±0.06	0.32±0.01	0.37±0.03	0.34 ^a ±0.03			
	120	0.07±0.01	0.13±0.01	0.07±0.01	0.07±0.01	0.08 ^b ±0.01			
	Mean	0.19 ^b ±0.02	0.21 ^{ab} ±0.02	0.21 ^{ab} ±0.0	0.24 ^a ±0.02				
POV (meq/kg)	0	1.16±0.10	1.30±0.05	1.29±0.04	1.29±0.09	1.26 ^d ±0.06	p>0.34	p<0.01	p0<.01
	30	1.75±0.04	1.70±0.02	1.81±0.03	1.87±0.01	1.78 ^c ±0.02			
	60	2.40±0.05	2.66±0.02	2.53±0.02	2.64±0.02	2.56 ^b ±0.03			
	120	4.02±0.10	3.78±0.05	3.55±0.05	3.35±0.11	3.67 ^a ±0.07			
	Mean	2.33 ^a ±0.07	2.36 ^a ±0.03	2.29 ^a ±0.03	2.29 ^a ±0.06				
TBARS (mg-MA/kg)	0	0.09±0.00	0.13±0.02	0.12±0.00	0.12±0.00	0.11 ^c ±0.00	p<0.01	p<0.01	p<0.01
	30	0.32±0.01	0.37±0.04	0.43±0.02	0.42±0.00	0.38 ^b ±0.02			
	60	0.36±0.04	0.46±0.00	0.57±0.01	0.45±0.00	0.46 ^a ±0.01			
	120	0.11±0.00	0.10±0.00	0.11±0.00	0.11±0.00	0.11 ^c ±0.00			
	Mean	0.23 ^c ±0.01	0.27 ^b ±0.01	0.31 ^a ±0.00	0.27 ^b ±0.00				

Variations in superscripts across treatment groups and interval days are statistically significant (p<0.05). T₀ is the group under control, T₁ is a 3 kGy gamma ray, T₂ is a 5 kGy gamma ray, and T₃ is a 7 kGy gamma ray. DI stands for Days of Interval, Treat for Treatment, and T*DI for Treatment-Interaction with Days of Interval.

The current study supports the findings of past investigations. They found that storage duration and radiation dosages increased oxidation activity and lipid peroxidation (Bachir and Zeinou, 2009; Bakalivanova et al., 2009). The highest TBARS levels measured in 60 days of interval. (Zhang et al., 2016) noted that when irradiation dosages increased, so did lipid oxidation. The rise in TBARS levels might be driven by lipid oxidation. The breakdown of peroxides and interaction with nucleophilic molecules, as well as carbonyl synthesis and irradiation, produced hydroxyl radicals, which accelerated lipid oxidation (Aubourg et al., 2004). (Park et al., 2010) and (Hocaoglu et al., 2012) discovered that the higher the irradiation dosages, the higher the TBARS readings in beef sausages.

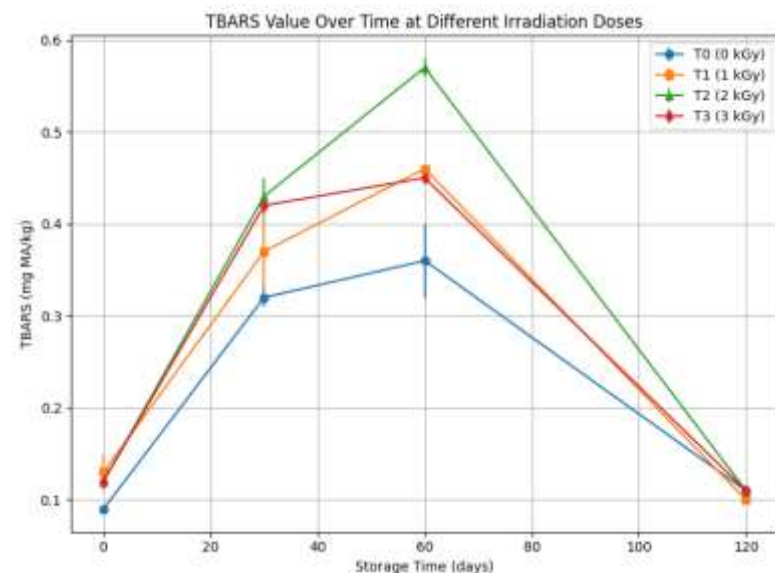


Figure 1. Effect of storage time and irradiation dose on TBARS values in meat samples. Error bars represent standard deviation. Significant differences observed among treatments (p<0.01).

Effect on microbial population

Total *Salmonella* species, TVC, TYMC, and total *Staphylococcus* species counts are presented in Table 2.

Table 2. Effect on microbial population of beef

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	1.61±0.05	1.65±0.03	1.63±0.02	1.70±0.09	1.64 ^a ±0.8	p>0.89	p<0.01	p>0.95
	30	1.53±0.03	1.47±0.08	1.47±0.04	1.48±0.07	1.49 ^b ±0.03			
	60	1.46±0.06	1.50±0.07	1.44±0.06	1.45±0.10	1.46 ^b ±0.05			
	120	1.22±0.05	1.25±0.09	1.33±0.08	1.35±0.05	1.28 ^c ±0.07			
	Mean	1.46 ^a ±0.04	1.47 ^a ±0.06	1.46 ^a ±0.05	1.49 ^a ±0.07				
TVC (logCFU/g)	0	3.05±0.07	3.30±0.07	3.15±0.16	3.56±0.06	3.35 ^a ±0.08	p<0.01	p<0.01	p>0.21
	30	2.46±0.13	2.69±0.05	2.75±0.11	2.76±0.16	2.66 ^b ±0.09			
	60	1.87±0.03	1.99±0.03	2.10±0.07	2.28±0.09	2.06 ^c ±0.02			
	120	1.88±0.04	1.81±0.06	1.87±0.02	1.84±0.05	1.85 ^d ±0.04			
	Mean	2.31 ^c ±0.06	2.45 ^{bc} ±0.05	2.55 ^{ab} ±0.09	2.61 ^a ±0.08				
TYMC (logCFU/g)	0	2.01±0.10	2.40±0.10	2.32±0.08	2.41±0.04	2.28 ^a ±0.06	p<0.01	p<0.01	p>0.14
	30	1.86±0.08	1.81±0.03	1.80±0.01	2.29±0.33	1.94 ^b ±0.08			
	60	1.71±0.04	1.77±0.05	1.69±0.12	1.67±0.09	1.71 ^c ±0.07			
	120	1.42±0.09	1.64±0.04	1.68±0.02	1.70±0.02	1.61 ^c ±0.03			
	Mean	1.75 ^b ±0.07	1.91 ^{ab} ±0.05	1.87 ^{ab} ±0.10	2.02 ^a ±0.12				
Salmonella (logCFU/g)	0	3.37±0.14	3.51±0.19	3.67±0.27	3.67±0.23	3.56 ^c ±0.20	p<0.01	p<0.01	p<0.01
	30	3.53±0.06	4.03±0.03	0.00±0.00	4.10±0.05	2.91 ^{bc} ±0.30			
	60	3.47±0.05	3.87±0.05	3.69±0.05	4.17±0.05	3.80 ^b ±0.05			
	120	4.60±0.00	4.22±0.20	4.43±0.00	3.54±0.00	4.19 ^a ±0.00			
	Mean	3.74 ^b ±0.06	3.90 ^a ±0.12	2.94 ^a ±0.08	3.87 ^a ±0.08				
Staphylococcus (logCFU/g)	0	3.67±0.17	3.67±0.12	3.53±0.18	3.58±0.23	3.61 ^a ±0.17	p<0.01	p<0.01	p<0.01
	30	4.47±0.05	3.65±0.00	3.60±0.05	3.80±0.07	3.88 ^a ±0.22			
	60	3.94±0.02	4.00±0.05	3.87±0.00	3.60±0.00	3.83 ^a ±0.00			
	120	4.74±0.00	3.58±0.14	3.65±0.00	3.69±0.00	3.91 ^a ±0.30			
	Mean	4.20 ^a ±0.06	3.73 ^b ±0.07	3.66 ^b ±0.05	3.66 ^b ±0.07				

Significant differences (p<0.05) exist in the superscripts of various treatment groups and interval days. T₀ stands for the control group, T₁ for a 3 kGy gamma ray, T₂ for a 5 kGy gamma ray, and T₃ for a 7 kGy gamma ray. T*DI = Interaction of Treatment and Days of Interval, DI = Days of Interval, and Treat = Treatment

The study found average values of TCC, TVC, TYMC, *Salmonella* spp., and total *Staphylococcus* spp. in various groups of treatment, with log CFU/g values ranging from 1.46 to 3.66. *Staphylococcus* species, TVC, TYMC, and *Salmonella* species all exhibited substantial (p<0.01) variations between different treatments and different storage durations, although TCC did not. Only *Salmonella* spp. and *Staphylococcus* spp. demonstrated significant treatment-time interaction. Compared to the control group, the levels of *Staphylococcus* and TYMC in the various groups given treatment were substantially reduced (p<0.05). *Staphylococcus* considerably (p<0.01) decreased and *Salmonella* insignificantly (p>0.05) increased as beef was exposed to increasing radiation levels. (Haque et al., 2017) proved similar results. *Salmonella* spp. dramatically reduced (p<0.01) and *Staphylococcus* spp. displayed significantly decreased (p<0.01) with increasing radiation dosages. (Ham et al., 2017) revealed that the entire aerobic bacteria from cooked beef patties was totally eradicated by electron beam irradiation levels of 2.5, 5, 7.5, and 10 kGy. It was found that as the irradiation dose increased in beef, there was a decreasing trend in TCC, TVC, and TYMC levels. TCC, TVC, and TYMC values in our study increased in groups receiving treatment because of higher irradiation dosages, but decreased throughout storage times (Islam et al., 2022; Haque et al., 2017).

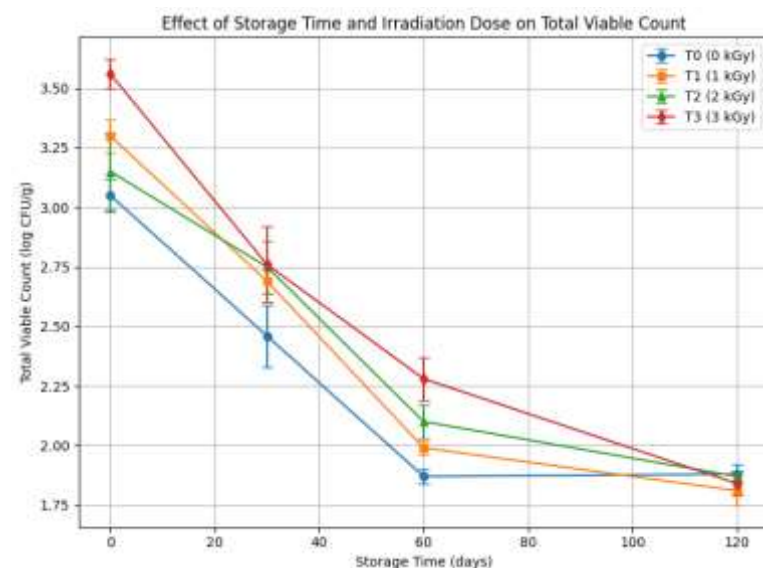


Figure 2. Total Viable Count (TVC) changes over storage time for different irradiation doses. Error bars represent standard deviation. Significant effects of dose and storage time observed (p<0.01).

Conclusion

BHA treatment combined with freezing influenced beef quality by accelerating lipid oxidation, as seen through increased FFA and TBARS and reduced POV over time. While microbial counts like TCC, TVC, and TYMC rose initially in irradiated samples, they declined with extended storage and stayed within safe limits. *Staphylococcus* spp. significantly decreased with higher irradiation, whereas *Salmonella* spp. showed a slight upward trend. The interaction between dose and storage time had a notable impact on most quality indicators. Overall, effective dose and storage control are essential to balance oxidative stability and microbial safety in treated beef.

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