

Enhancing Shelf Life and Quality of Traditional Thai Pork Sausage (Num-Tub) Using Gamma Irradiation

Jaruratana Eamsiri*, Ratchaneeporn Photinam, Sirilak Chookaew,
Khemruji Khemthong, Surasak Sajjabut and Wachiraporn Pewlong

Nuclear Technology Research and Development Center, Thailand Institute of Nuclear Technology (Public Organization),
Nakhon Nayok 26120, Thailand

(*Corresponding author's e-mail: jaruratana@tint.or.th)

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Abstract

Num-Tub is a traditional Northeastern Thai pork sausage, often consumed fresh or fermented. The ingredients were naturally rich in protein, fat and moisture content. These conditions are conducive to the growth of beneficial and harmful microbes. Its perishable nature poses microbial safety challenges. Irradiation effectively extends the shelf life of Num-tub by reducing microbial contamination and inactivating spoilage microorganisms. This study investigated the effects of gamma irradiation at doses of 0, 5, and 10 kGy on the microbiological, chemical, and color attributes of vacuum-packed Num-Tub during 24 days at 28 ± 2 °C. Irradiation at 5 kGy reduced total viable counts (TVC) and lactic acid bacteria (LAB) to undetectable levels, though microbial regrowth occurred over time and eliminated key pathogens including *Salmonella* spp, *E. coli*, *C. perfringens*, *S. aureus*. However, this dose could not prevent microbial regrowth. In contrast, irradiation at 10 kGy improved microbial control and color (higher oxymyoglobin and redness) up to day 9 but significantly increased lipid oxidation. Correlation analysis indicated positive associations between microbial growth, acidity, lipid oxidation, and metmyoglobin formation, while pH, myoglobin, oxymyoglobin, and redness were negatively correlated. Gamma irradiation at 10 kGy is a non-thermal method that improves microbial safety by eliminating pathogens, suppressing lactic acid. It also increases oxymyoglobin levels, which enhances product redness. However, it may also lead to increased lipid oxidation during the 9-day storage period at ambient temperature. Further research should focus on integrating gamma irradiation with natural antioxidants or modified atmosphere packaging to mitigate lipid oxidation and pigment degradation. Additionally, sensory evaluation and consumer acceptance studies are essential to establish optimal irradiation protocols that balance safety, quality, and market viability.

Keywords: Microbial safety, Gamma irradiation, Lipid oxidation, Color, Myoglobin, Shelf life, Isan-style sausage

Introduction

Meat and meat products are recognized as major contributors to foodborne illness outbreaks [1]. Fermented meat products are highly susceptible to microbial contamination, and outbreaks linked to fermented sausages have been reported in several countries [2]. Abuelnaga *et al.* [3] found that minced meat exhibited the highest total microbial counts, sausage samples contained the greatest levels of *Staphylococcus aureus*, luncheon meat was most frequently contaminated with *Escherichia coli*, and

Salmonella spp. were most abundant in minced meat. Traditional fermented sausages are especially vulnerable because pathogens can be introduced through raw ingredients, equipment, or handling during processing [4]. With rising global demand for processed foods, microbial contamination has become a critical concern, causing an estimated 25% loss of processed products annually due to spoilage [5]. These challenges point to the urgency of developing preservation strategies that improve microbial safety and extend shelf

life while preserving the sensory qualities and cultural significance of traditional foods such as Num-tub.

Num-tub, a traditional Thai sausage from Nakhon Ratchasima Province, represents the intersection of local identity, culinary resilience, and community heritage, reflecting the distinctive food culture of the region. Its preparation showcases preservation techniques rooted in rural life, involving the stuffing of ground pork mixed with finely chopped liver or spleen and spices into natural casings such as pork intestines or stomach. The sausages are typically air- or sun-dried, which imparts a mildly sour flavor that defines their regional character. This product contains high levels of fat, protein, and moisture, which provide an ideal environment for microbial growth. As a result, it is highly prone to spoilage and poses a significant risk of foodborne illness. Currently, Num-tub is distributed in raw form and requires frozen transportation to minimize spoilage and ensure microbial safety. However, this product distribution was limited because of the storage condition. Freezing storage was a significant financial burden for small-scale producers and local manufacturers.

Food irradiation has been recognized as a safe and effective technology for enhancing food safety and extending shelf life. Three types of ionizing radiation such as gamma rays, X-rays, and electron beams are approved for use in food processing. The appropriate type of radiation based on the food type, desired shelf-life extension, and specific processing requirements [6,7]. Ionizing radiation works by damaging the DNA and essential cellular components of microorganisms, thereby preventing replication and leading to cell death [8]. Among the available methods, gamma rays possess the highest penetration power and stable energy levels, making them particularly suitable for dense or thick food products. Gamma irradiation has emerged as a promising non-thermal preservation technique for meat and meat products [9], suitable for Num-tub, as it does not induce heat-related changes. This method effectively reduces microbial contamination, inhibits spoilage and pathogenic microorganisms, and extends shelf life without compromising the product's structural integrity [9,10-12]. However, irradiation may also trigger chemical alterations, including lipid oxidation and protein denaturation, which can diminish nutritional value and negatively affect the natural appearance and

sensory attributes of the product [13-16]. Therefore, it is essential to investigate the optimal radiation dose, evaluate its impact on product quality, and explore complementary techniques to mitigate undesirable effects and enhance consumer acceptance.

In several Asian countries, such as Bangladesh, China, and the Republic of Korea, irradiated food products have been successfully commercialized and widely distributed, accompanied by consumer education initiatives that have fostered public acceptance. Sri Lanka has achieved effective spice decontamination through irradiation, although consumer acceptance remains relatively low. In contrast, Thailand and Viet Nam have either commercialized or piloted irradiated products, receiving positive consumer responses and showing increasing adoption within the food industry [17]. Especially in Thailand, a market trial of irradiated Nham demonstrated that Nham treated with 2 kGy was accepted by consumers as microbiologically safe, with taste, texture, and aroma remaining at satisfactory levels. This product has since progressed to commercial distribution [18].

This research focuses on a traditional Thai sausage (Num-tub) that contains nutrient-rich composition. Due to its nutrient-rich composition and high moisture content, the product provides an ideal environment for microbial growth. Furthermore, its raw form of distribution renders heat-based preservation methods unsuitable. Gamma irradiation presents a viable non-thermal alternative for preserving meat products, offering effective microbial reduction and shelf-life extension [9]. This study was undertaken with the hypothesis that gamma irradiation could effectively improve the microbial safety and storage stability of Num-tub. In addition, it aimed to comprehensively assess the impact of irradiation on the physical and chemical characteristics of this product. The goal is to enable ambient-temperature storage, thereby facilitating safer distribution and improving accessibility to this culturally significant heritage food.

Materials and methods

Gamma irradiation

Vacuum-packed frozen Num-Tub pork sausage samples were procured from a certified manufacturer located in Nakhon Ratchasima province, Thailand. This product is composed of 75% minced pork with fat, 11%

garlic, 2% ground black pepper, 4% seasoning powder, 3.5% monosodium glutamate (MSG), 4% salt, and 0.5% sodium nitrite. Gamma irradiation was performed at doses of 5 and 10 kGy using a Co-60 gamma irradiator (Ignis, Hungary) operated at the Thailand Institute of Nuclear Technology (Public Organization), with a dose rate of 8.65 kGy/h. The absorbed dose delivered to each sample was quantified using an amber Perspex dosimeter composed of polymethylmethacrylate (PMMA), ensuring accurate dose verification. Both irradiated and non-irradiated samples were stored at ambient temperature (28 ± 2 °C) and periodically analyzed to investigate changes in physicochemical and microbiological properties throughout the storage period.

Sample preparation

All samples for microbiological and chemical analysis were prepared following a similar procedure. A composite sample was prepared by randomly selecting 5 individual bags (100 g each) and homogenizing them thoroughly. However, sample preparation for microbiological parameters must be prepared under aseptic conditions.

Total Viable Count (TVC)

Each sample was prepared a serial dilution with phosphate-buffered solution. One mL of appropriated dilutions was inoculated into petri dishes in triplicate. After that, we poured Plate Count Agar (PCA) into the dishes, gently mixed the contents, allowed the medium to solidify. The plates were incubated at 35 ± 1 °C for 48 ± 2 h. After incubation, we counted colony-forming units (CFU) ranging from 25 to 250 per plate and used the results to calculate aerobic plate counts according to AOAC Official Method 966.23 [19].

Total yeast and mold count (TYM)

Yeast and molds were enumerated using a pour plate method. Irradiated and non-irradiated samples were homogenized in aseptic technique. Then prepared a serial dilution in Butterfield's Phosphate Buffer (BPB) to the appropriate dilution. Aliquots of 1 mL from appropriate dilutions were plated in triplicate and incubated at 20 - 25 °C for 3 - 5 days. Colonies were counted and results expressed as colony-forming units per gram (CFU/g) [20].

Enumeration of *Clostridium perfringens*.

Enumeration of *C. perfringens* was conducted following ISO 7937:2004. For each sample, we homogenized 10 g of sample in 90 mL of sterile diluent and performed serial dilutions. We plated appropriate dilutions on Tryptose Sulphite Cycloserine (TSC) agar in triplicate and incubated anaerobically at 37 °C for 24 ± 2 h. After incubation, we counted characteristic black colonies and reported the results as colony-forming units per gram (CFU/g) [21].

Enumeration of *Escherichia coli*.

We enumerated *E. coli* following FDA-BAM Chapter 4 (2020). We serially diluted the samples and inoculated them into Lauryl Sulfate Tryptose (LST) broth at 35 °C for presumptive coliform detection. We transferred gas-positive tubes to EC broth and incubated them at 44.5 °C to confirm the presence of *E. coli*. For final confirmation, we performed the LST-MUG fluorescence assay. We expressed the results as the most probable number per gram of sample (MPN/g) [22].

Detection of *Staphylococcus aureus*.

We pre-enriched a 25 g portion of each sample in broth and streaked it onto Baird-Parker Agar (BPA). We incubated the plates at 37 ± 1 °C for 24 - 48 ± 2 h. After incubation, we transferred both typical and atypical colonies to Brain Heart Infusion (BHI) broth and incubated them again at 37 ± 1 °C for 24 ± 2 h. We tested the resulting isolates for coagulase activity. Isolates showing 1+/2+ or 3+/4+ reactions were further examined for thermonuclease activity. We confirmed thermonuclease-positive isolates as *S. aureus*, while we excluded the negative ones [23].

Detection of *Salmonella* spp

We performed a pre-enrichment step, followed by selective enrichment using Rappaport-Vassiliadis (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broths. We incubated the RVS broth at 41.5 ± 1 °C and the MKTTn broth at 37 ± 1 °C for 24 ± 3 h. After enrichment, we streaked the cultures onto Xylose Lysine Deoxycholate (XLD) and Hektoen Enteric (HE) agars and incubated the plates at 37 ± 1 °C for 24 ± 3 h. We subculture the typical colonies onto Nutrient Agar. We confirmed presumptive isolates using biochemical tests and auto-agglutination. The

Department of Medical Sciences, Thailand, verified the final identification [24].

Mesophilic lactic acid bacteria

We enumerated mesophilic lactic acid bacteria following ISO 15214:1998. We serially diluted the samples in sterile diluent and inoculated 1 mL aliquots into Petri dishes containing de Man, Rogosa, and Sharpe (MRS) agar adjusted to pH 5.7. We incubated the plates at 30 ± 1 °C for 72 ± 3 h under aerobic conditions. After incubation, we counted colonies ranging from 15 to 300 CFU per plate and expressed the results as colony-forming units per gram of sample (CFU/g) [25].

pH measurement

The pH of samples was measured according to AOAC (2019). Ten g of sample were homogenized with 100 mL distilled water. The mixture was allowed to equilibrate at room temperature for 5 min before measuring. The pH value was recorded in triplicate with a calibrated digital pH meter standardized with pH 4.0 and 7.0 buffers [26].

Titrateable acidity as lactic acid

We determined titrateable acidity following AOAC (2019). We homogenized 10 g of the sample with 100 mL of distilled water and titrated the mixture using 0.1 N NaOH. We used phenolphthalein as an indicator and continued titration until a faint pink endpoint appeared. We calculated the percentage of lactic acid based on the volume of NaOH consumed and expressed the results as percent lactic acid (% lactic acid) [27].

Thiobarbituric acid (TBA)

Thiobarbituric acid reactive substances (TBARS) were performed following the method of Tarladgis *et al.* [28]. Briefly, 10 g of homogenized meat sample was mixed with distilled water and acidified with hydrochloric acid prior to distillation. A 50 mL distillate was collected and reacted with 0.02 M thiobarbituric acid solution. The mixture was heated in a boiling water bath for 35 min, cooled to room temperature, and the absorbance was measured at 532 nm using a spectrophotometer. The results were expressed as milligrams of malondialdehyde (MDA) per kilogram of sample.

Measurement of the relative content of myoglobin

The proportions of myoglobin derivatives were determined using a modified Krzywicke method. We homogenized approximately 5 g of each sample with 25 mL of 40 mM phosphate buffer (pH 6.8) at 10,000 rpm for 25 s. We kept the homogenates in the dark at 4 °C for 60 min and then centrifuged them at $4,500 \times g$ for 20 min. We filtered the resulting supernatant and measured absorbance at 525, 545, 565, and 572 nm using a spectrophotometer. We calculated the relative contents of myoglobin (Mb), oxymyoglobin (MbO₂), and metmyoglobin (MetMb) from absorbance ratios using the equations proposed by Krzywicke [29].

The formulas used were as follows:

$$P_1 = (0.369R_1 + 1.140R_2 - 0.941R_3 + 0.015) \times 100 \quad (1)$$

$$P_2 = (0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361) \times 100 \quad (2)$$

$$P_3 = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100 \quad (3)$$

where P_1 , P_2 , and P_3 represent the mass fractions of Mb, MbO₂, and MetMb, respectively, and $R_1 = A_{572}/A_{525}$, $R_2 = A_{565}/A_{525}$, $R_3 = A_{545}/A_{525}$.

Measurement of Color (L, a, b)

Irradiated and non-irradiated samples were determined the color alteration using Colorimeter (ColorFlex® EZ, HunterLab Co., Ltd., USA.). This colorimeter was calibrated with standard black and white tile before measurement. The color of sample showed into tree dimension color scale which L measuring light to dark color components (0 = black, 100 = white), a is a red (-) to green (+) scale and b is a yellow (+) to blue (-) scale.

Statistical analysis

All statistical evaluations were performed using SPSS software (version 29.0, IBM Corp., Armonk, NY, USA). Determination of the significance variances between mean values, ANOVA and Duncan's multiple-range tests were conducted. Each analysis was performed in triplicate, and the statistical significance level was set at 5% ($p < 0.05$).

Results and discussion

Effect of gamma irradiation on microbial quality

The effect of gamma irradiation on the microbial quality of pork sausage (Num-Tub) during storage is summarized in **Table 1**. At day 0, the non-irradiated control exhibited a high Total Viable Count (TVC) of 8.7×10^6 CFU/g, whereas irradiation at 5 and 10 kGy effectively reduced the microbial population to below the detection limit (< 10 CFU/g). Pathogenic indicators, including *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., were absent in all irradiated samples, while *Salmonella* spp. was detected in the non-irradiated control. During storage, microbial proliferation was evident in the control samples, with TVC increasing to 1.6×10^7 CFU/g by day 3. In contrast, irradiated samples maintained significantly lower bacterial counts. At 5 kGy, TVC began to reappear by day 3 (8.3×10^3 CFU/g) and increased sharply during storage, reaching 2.2×10^8 and 1.5×10^8 CFU/g on days 6 and 9, respectively. Samples treated with 10 kGy effectively inhibited microbial regrowth, with bacterial counts remaining very low (30 - 80 CFU/g) up to day 6. Although counts gradually increased during extended storage, they consistently remained lower than those in the 5 kGy treatment; for example, bacterial loads were 3.2×10^3 CFU/g on day 9, 4.7×10^5 CFU/g on day 12, and reached 3.5×10^7 CFU/g by day 24. Yeast and mold remained below detectable levels (< 10 CFU/g) throughout most of the storage period, with occasional detections (10 CFU/g) in 10 kGy-treated samples after day 12. Microbial regrowth was observed during extended storage beyond day 12 in the 10 kGy treatment, increasing from 4.7×10^5 CFU/g on day 12 to 3.5×10^7 CFU/g by day 24. Importantly,

pathogenic bacteria, including *C. perfringens*, *E. coli*, *S. aureus*, and *Salmonella* spp., were not detected in any irradiated samples throughout the 24-day storage period. These results confirm that gamma irradiation effectively suppresses microbial growth and maintains pathogen-free products during storage. Setyawan *et al.* [30] conducted an experiment using gamma irradiation on tuna intended for sushi or sashimi at doses of 0, 2, and 4 kGy, combined with cold storage for 1 - 3 days. The results showed that irradiation at 4 kGy was the most effective in reducing microbial contamination, lowering the Total Viable Plate Count and Coliform bacteria by approximately 3 log cycles. The microbial load decreased proportionally with increasing radiation dose. Previous studies on beef and chicken have demonstrated that both irradiation dose and temperature significantly influence microbial inactivation, with doses of approximately 0.3 - 0.4 kGy achieving a 90% reduction of *E. coli* O157:H7 [31]. Gamma irradiation causes microbial damage through both direct and indirect mechanisms. Directly, it damages genetic material and disrupts cell division, leading to cell death or the inability to reproduce. Indirectly, radiation interacts with water molecules, triggering radiolytic reactions that generate free radicals. Subsequently, these free radicals attack cytoplasmic components and destroy organic molecules. The hydroxyl free radical removes hydrogen atoms from the bases within the DNA strands, causing death to organisms which cannot recover from this DNA damage [32]. Thus, gamma irradiation represents a reliable non-chemical preservation method, although the irradiation dose must be carefully selected to ensure microbial safety without compromising physicochemical or sensory quality.

Table 1 Effect of gamma irradiation at 0, 5, and 10 kGy on microbial quality of Num-Tub pork sausage during 24 days at ambient storage.

Storage time (days)	Irradiation dose (kGy)	Total viable count (CFU/g)	Yeast & Mold (CFU/g)	<i>C. perfringens</i> (CFU/g)	<i>E. Coli</i> (MPN/g)	<i>S. aureus</i> (CFU/g)	<i>Salmonella</i> spp. (per 25 g)
0	0 (Control)	8.70×10^6	< 10	< 10	< 3	< 10	DT
	5	< 10	< 10	< 10	< 3	< 10	ND
	10	< 10	< 10	< 10	< 3	< 10	ND
3	0 (Control)	1.60×10^7	< 10	ND	ND	ND	ND
	5	8.30×10^3	< 10	ND	ND	ND	ND
	10	30	< 10	ND	ND	ND	ND

Storage time (days)	Irradiation dose (kGy)	Total viable count (CFU/g)	Yeast & Mold (CFU/g)	<i>C. perfringens</i> (CFU/g)	<i>E. Coli</i> (MPN/g)	<i>S. aureus</i> (CFU/g)	<i>Salmonella</i> spp. (per 25 g)
6	5	2.20×10 ⁸	< 10	ND	ND	ND	ND
	10	80	< 10	ND	ND	ND	ND
9	5	1.50×10 ⁸	< 10	ND	ND	ND	ND
	10	3.20×10 ³	< 10	ND	ND	ND	ND
12	10	4.70×10 ⁵	10	ND	ND	ND	ND
15	10	1.60×10 ⁸	10	ND	ND	ND	ND
18	10	4.70×10 ⁶	< 10	ND	ND	ND	ND
21	10	4.00×10 ⁶	10	ND	ND	ND	ND
24	10	3.50×10 ⁷	< 10	ND	ND	ND	ND

Notes: Mean ± standard deviation (n = 3). Different superscript letters within the same storage day indicate significant differences ($p < 0.05$). ND = Not Detected and DT = Detected

Effect of gamma irradiation on lactic acid bacteria (LAB), acidity, and pH

The effects of gamma irradiation on lactic acid bacteria (LAB), acidity, and pH during storage are summarized in **Table 2**. On day 0, non-irradiated samples exhibited LAB counts of 5.70×10^6 CFU/g, whereas irradiation at 5 and 10 kGy reduced LAB to below the detection limit (< 10 CFU/g). In raw fermented sausages such as Num-Tub, LAB are essential for flavor development and microbial safety, as heterofermentative strains convert fermentable sugars into lactic acid, acetic acid, ethanol, and carbon dioxide [33]. Higher irradiation doses effectively suppressed microbial activity, delaying acid production and influencing acid-base dynamics during storage. Acidity in irradiated samples was lower (0.33% - 0.36%) compared to the control (0.54%), while pH was higher (6.52 - 6.71 vs. 5.95). By day 3, microbial growth became evident in non-irradiated samples, with LAB increasing to 1.40×10^8 CFU/g, accompanied by higher acidity (1.11%) and lower pH (4.85). In contrast, irradiated samples maintained lower microbial counts: LAB in the 5 kGy and 10 kGy treatments were 8.50×10^3 and 50 CFU/g, respectively, with correspondingly higher pH values, reflecting reduced acidification intensity. Higher irradiation doses effectively suppressed microbial activity, delaying acid production and influencing acid-base dynamics during storage. Fluctuations in pH at higher doses may reflect partial microbial recovery or chemical buffering from protein and carbohydrate breakdown [34]. After day 6, rapid LAB growth occurred in the 5 kGy samples (3.40×10^8

CFU/g), with increased acidity (1.17% - 1.18%) and decreased pH (< 4.6). The 10 kGy treatment effectively suppressed microbial proliferation, maintaining LAB below 10 CFU/g at day 6, with a slight increase to 3.00×10^3 CFU/g by day 9. Corresponding acidity and pH values ranged from 0.66% to 0.88% and 4.91 to 6.00, respectively. Microbial regrowth in 10 kGy-treated samples was observed by day 12 (LAB 2.60×10^5 CFU/g), accompanied by increasing acidity and decreasing pH. Extended storage (days 12 - 24) showed gradual increases in LAB (2.60×10^5 to 1.20×10^8 CFU/g), progressive acidification (up to 1.38%), and pH decline (to 4.39). Minor decreases in LAB activity toward the end of storage likely resulted from inhibitory effects of low pH. Previous research indicates that optimal pH conditions vary among LAB strains; for example, *Leuconostoc mesenteroides* 67-1, *Streptococcus infantarius* 46-3, and *Lactobacillus plantarum* 60-1 exhibit optimal growth at $\text{pH } 4.5 \pm 0.5$, 5.5 ± 0.5 , and 6.0 ± 0.5 , respectively [25]. Gamma irradiation effectively suppressed microbial growth and delayed acidification, with the 10 kGy dose providing the most effective control. It maintained low microbial counts and higher pH for up to 9 days, whereas the 5 kGy dose offered only short-term inhibition, with rapid regrowth observed after day 6. These findings highlight the dual role of LAB in fermented meats. While LAB contribute to desirable acidification and flavor development, they may also cause spoilage if their growth becomes excessive. Suppression of LAB in 10 kGy-treated samples slowed acidification, as reflected by relatively higher pH values. This finding is consistent

with previous studies demonstrating that gamma irradiation can modulate LAB activity and delay fermentation kinetics in meat products. Gamma irradiation at 10 kGy offers a balanced approach, reducing initial LAB load while allowing controlled regrowth to support fermentation without compromising shelf life. These results underscore the dual role of LAB in fermented meats: Contributing to desirable

acidification and flavor but potentially causing spoilage if overgrown [35]. Gamma irradiation at 10 kGy effectively reduces initial LAB levels while permitting controlled regrowth for fermentation, ensuring shelf stability and offering a practical microbial control strategy for traditionally fermented sausages stored at ambient temperature.

Table 2 Effect of gamma irradiation at 0, 5, and 10 kGy on lactic acid bacteria (LAB), acidity, and pH values of Num-tub during 24 days at ambient temperature.

Storage time (day)	Irradiation doses (kGy)	Parameters		
		LAB (CFU/g)	Acidity (g/100 g)	pH
0	Non-irradiation	5.70×10^6	0.54 ± 0.01^a	5.95 ± 0.01^c
	5	< 10	0.36 ± 0.02^b	6.52 ± 0.02^b
	10	< 10	0.33 ± 0.04^c	6.71 ± 0.01^a
3	Non-irradiation	1.40×10^8	1.11 ± 0.03^a	4.85 ± 0.03^c
	5	8.50×10^3	0.69 ± 0.02^b	5.39 ± 0.01^b
	10	50	0.62 ± 0.03^c	5.73 ± 0.01^a
6	5	3.40×10^8	1.17 ± 0.02^a	4.57 ± 0.01^b
	10	< 10	0.66 ± 0.02^b	6.00 ± 0.02^a
9	5	5.60×10^8	1.18 ± 0.02^a	4.44 ± 0.01^b
	10	3.00×10^3	0.88 ± 0.02^b	4.91 ± 0.02^a
12	10	2.60×10^5	1.21 ± 0.03	4.74 ± 0.05
15	10	7.30×10^7	1.38 ± 0.03	4.52 ± 0.02
18	10	4.20×10^7	1.28 ± 0.03	4.58 ± 0.02
21	10	3.50×10^7	1.01 ± 0.03	5.22 ± 0.02
24	10	1.20×10^8	1.35 ± 0.01	4.39 ± 0.02

Notes: Mean \pm standard deviation (n = 3). Different superscript letters within the same storage day indicate significant differences ($p < 0.05$).

Gamma irradiation on lipid oxidation (TBA Value)

The thiobarbituric acid (TBA) values, indicating lipid oxidation, of traditional pork sausage (Num-Tub) during storage under different gamma irradiation doses are shown in **Figure 1**. TBA value is commonly used as an index of lipid oxidation in meat products. TBA values reflect the amount of malondialdehyde (MDA), a major byproduct of fat breakdown responsible for rancidity, off-flavors, and reduced meat quality. At day 0, the non-irradiated control had a TBA value of 0.32 mg MDA/kg, while samples irradiated at 5 and 10 kGy showed slightly higher values of 0.36 and 0.45 mg MDA/kg, respectively, indicating minimal initial

oxidation. During storage, TBA values of non-irradiated and both of irradiated samples showed a slight increase, reaching 0.79, 1.34 and 1.80 MDA/kg, respectively on day 3. On day 6, lipid oxidation in both of irradiated groups increased to 1.01 and 2.05 mg MDA/kg, respectively, indicating a progressive oxidative process. After day 6, TBA values of 10 kGy treatment was rapidly increased throughout storage, with 4.02 mg MDA/kg at day 9 and 4.12 mg MDA/kg at day 24. These results align with previous studies showing that higher irradiation doses promote oxidative changes in meat products [36]. Haque et al. (2017) [37] reported that gamma irradiation of raw beef (2 - 6 kGy) during storage at -20°C increased TBA, peroxide values, free fatty

acids, and cooking loss, while reducing ash content and pH. Similarly, Indiaro *et al.* [38] observed dose-dependent increases in malondialdehyde formation in irradiated meat. TBA values were influenced by irradiation dose and extended storage time. The increase in TBA values with gamma irradiation is attributed to free radical formation. These free radicals attack unsaturated fatty acids, leading to the formation of lipid radicals and subsequent peroxy radicals by reacting with oxygen. This chain reaction propagates, producing hydroperoxides and other secondary oxidation products like carbonyls, which contribute to rancidity and the

deterioration of meat quality. Over longer storage periods, hydroperoxides decompose into more stable but often undesirable secondary products, including aldehydes (like malondialdehyde) and ketones, which are responsible for rancid odors and flavors [39]. Gamma irradiation at 5 kGy effectively maintained lipid stability during 9 days of storage, while the higher doses extremely accelerate oxidation. These findings suggest that while 10 kGy irradiation offers superior microbial safety, careful utilization of antioxidant or controlled atmosphere packaging are necessary to minimize lipid oxidation and preserve product quality.

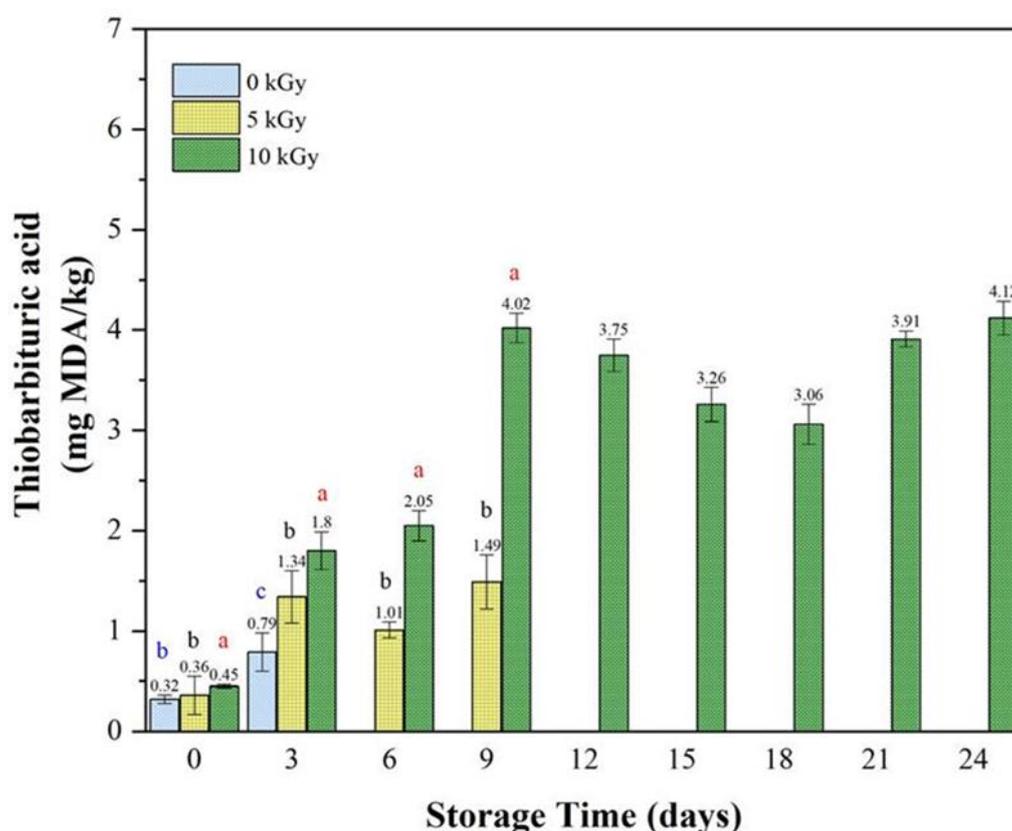


Figure 1 Thiobarbituric acid (TBA) values, expressed as malondialdehyde (MDA, mg/kg), in Num-tub sausages treated with gamma irradiation at 0, 5, and 10 kGy during 24 days of ambient storage (28 ± 2 °C). Data are presented as mean \pm SD ($n = 3$). Different letters above the bars indicate significant differences ($p < 0.05$) between treatments at the same storage time.

Myoglobin forms in Traditional Pork Sausage during storage

Meat color is primarily determined by myoglobin, an oxygen-binding muscle protein. Under low-oxygen conditions, myoglobin exists as deoxymyoglobin (Mb), giving a dark purplish-red hue, whereas exposure to oxygen converts Mb to oxymyoglobin (MbO₂), producing

the bright red color associated with freshness. Oxidation to metmyoglobin (MetMb) during storage results in brown discoloration, indicative of spoilage [40]. This study monitored Mb, MbO₂, and MetMb in gamma-irradiated Num-Tub samples (5 and 10 kGy) and non-irradiated controls over 24 days of ambient storage in vacuum packaging (**Figure 2**). On day 0 (**Figure 2(A)**),

non-irradiated samples contained 25.42% Mb, 9.26% MbO₂, and 61.14% MetMb. Gamma irradiation slightly increased Mb (27.92% at 10 kGy) and decreased MetMb (55.54%), while MbO₂ remained ~10%, with no significant differences ($p > 0.05$). These results are consistent with Nisar *et al.* (2020) [41], who reported similar changes in myoglobin forms during storage. On day 0, myoglobin, oxymyoglobin, and metmyoglobin levels ranged from 28.98% to 34.08%, 8.98% to 11.77%, and 42.12% to 45.05%, respectively. In 3 kGy chicken meat samples with MLP (moringa leaf powder), myoglobin was highest on day 0 (35.65%), while oxymyoglobin peaked at 20.61% in 3 kGy + MLP samples on day 14.

By day 3 of storage (**Figure 2(B)**), the non-irradiated samples showed a sharp decline in oxymyoglobin (MbO₂) levels to 1.22%, accompanied by an increase in metmyoglobin (MetMb) to 64.92%, indicating rapid myoglobin oxidation. In contrast, samples treated with gamma irradiation at doses of 5 - 10 kGy maintained higher MbO₂ levels (10.20% - 11.06%) and lower MetMb levels (55.14% - 57.61%), suggesting that irradiation effectively delayed oxidation during the early storage period and contributed to improved visual quality. Mechanistically, gamma irradiation promotes the conversion of myoglobin into both oxymyoglobin and metmyoglobin, with myoglobin acting as a prooxidant that generates free radicals, thereby accelerating oxidative processes [42]. Under low-oxygen conditions, irradiation may also facilitate the release of molecular oxygen from endogenous

compounds such as hydroperoxides, or through the radiolytic decomposition of water. The released oxygen can bind to deoxymyoglobin, forming oxymyoglobin and helping preserve the desirable bright red color of meat [43].

Between days 6 and 9 (**Figures 2(C)** and **2(D)**), MbO₂ in irradiated samples fluctuated (8.25% - 16.20%) with corresponding decreases in MetMb (49.37% - 61.72%), reflecting partial stabilization of the myoglobin redox state. In this study, myoglobin levels declined, whereas oxymyoglobin increased and metmyoglobin decreased with higher irradiation doses, a trend consistent with An *et al.* [44]. These findings suggest that gamma irradiation accelerates myoglobin oxidation, thereby affecting color stability during storage. Similarly, Reddy *et al.* [45] reported a decrease in myoglobin content accompanied by increases in oxymyoglobin and metmyoglobin during storage of irradiated mutton keema. These results indicate that gamma irradiation accelerates myoglobin oxidation, influencing color stability. Notably, 10 kGy-treated sausages retained higher MbO₂ and lower MetMb than 5 kGy samples, suggesting better red color preservation. During extended storage (days 12 - 24) of 10 kGy irradiated samples (**Figure 2(E)**), Mb and MbO₂ gradually decreased while MetMb increased, reflecting progressive oxidation. In the later stages of storage, an increase in metmyoglobin content is observed due to the depletion of internally generated oxygen, leading to the oxidation of oxymyoglobin into metmyoglobin [46].

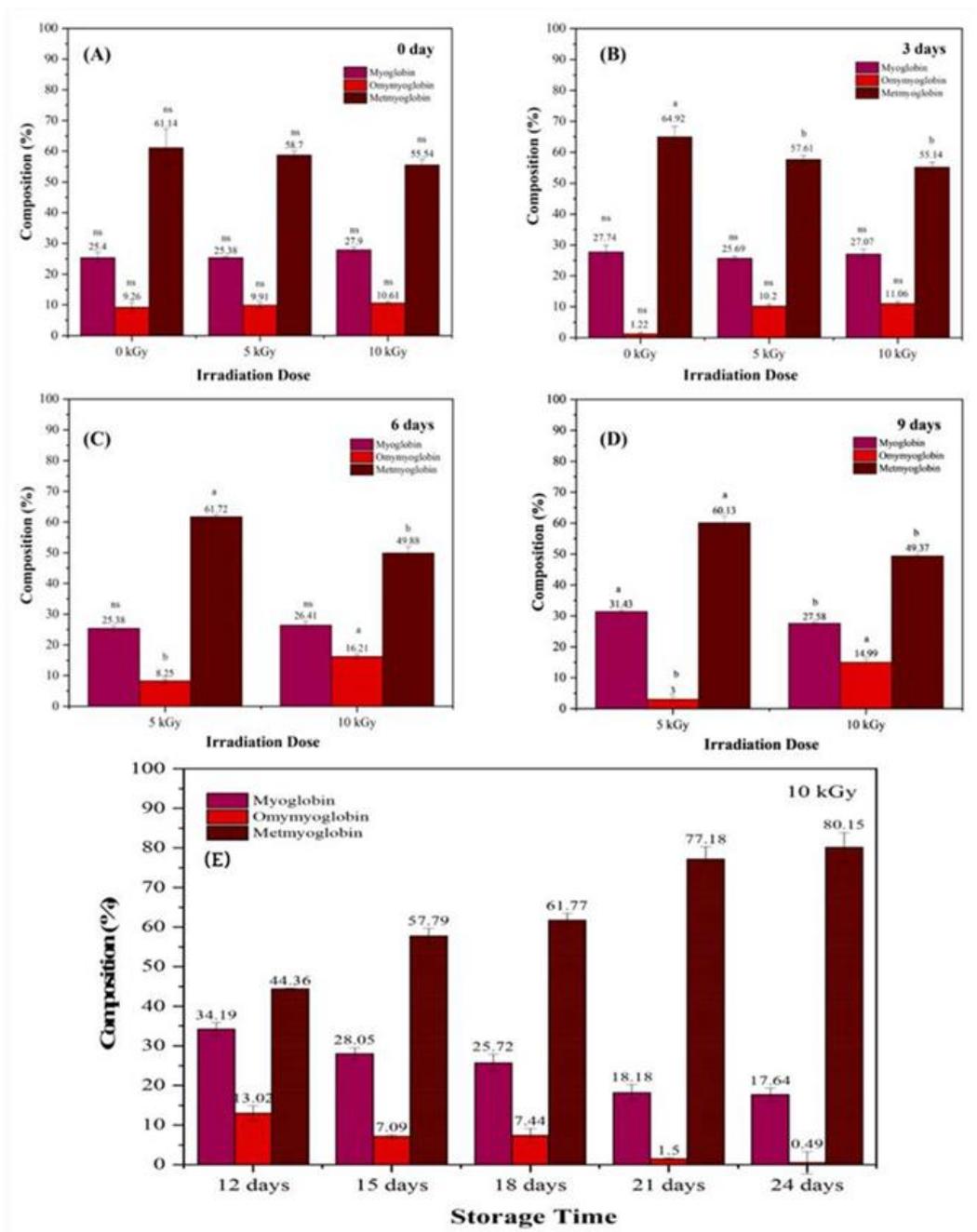


Figure 2 Effect of gamma irradiation (0, 5, and 10 kGy) on pigment composition (myoglobin, oxymyoglobin, and metmyoglobin) in Num-tub sausages during ambient storage (28 ± 2 °C). (A-B) show pigment composition at irradiation doses of 0, 5, and 10 kGy on (A) day 0 and (B) day 3. (C-D) show pigment composition at doses of 5 and 10 kGy on (C) day 6 and (D) day 9. (E) shows changes at 10 kGy from day 12 to day 24. Values are expressed as mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments at the same storage time, and “ns” denotes no significant difference.

Effect of gamma irradiation on color parameters

The color parameters (L, a, b) of Num-Tub pork sausage during storage at different gamma irradiation doses are summarized in **Table 4**. At day 0, gamma irradiation slightly darkened the sausages, with L values

decreasing from 46.52 in non-irradiated samples to 43.17 - 43.37 in 5 - 10 kGy-treated samples. Throughout storage, L values of irradiated samples remained slightly lower than the controls, indicating stable lightness over time. In meat products, the higher a value reflecting greater redness and freshness. Redness (a) increased

significantly immediately after irradiation (5.96 - 6.61 vs. 4.78 in controls, $p < 0.05$) and remained higher than controls through day 9, suggesting enhanced red coloration likely due to radiation-induced oxidation of myoglobin in meats. These observations are consistent with previous studies Triyannanto *et al.* [47]; Qadr *et al.* [48] increased a^* values in irradiated meat products because of oxidative pigment changes. Yellowness (b) was slightly reduced by 5 kGy at day 0 (10.24 ± 0.24) but remained similar across treatments (10.24 - 10.36). Throughout storage, it fluctuated within the range of 9.17 - 11.38 during storage, demonstrating minimal effect of irradiation on the yellow component. From days 12 - 24, 10 kGy-treated samples maintained stable color parameters (L 44.67 - 46.33, a 6.36 - 6.57, b 10.29 - 11.38), indicating minimal overall color degradation for 24 days of storage. After irradiation, radiolytic products such as CO_2 , CO , H_2 , hydrocarbons and aldehydes were produced. The red coloration in irradiated Num-tub remained stable throughout the 24

days of storage period. These experimental results may not align with the observed decrease in oxymyoglobin and the corresponding increase in metmyoglobin between days 9 and 24. This phenomenon may be attributed to the interaction between myoglobin and carbon monoxide (radiolytic products), which produces a similar red hue. In consistency with Nam and Ahn [49] reported that the CIE a^* of raw turkey meat increased after 4.5 kGy electron beam irradiation because it can produce carbon monoxide in meat. When CO binds to myoglobin, carboxymyoglobin (CO-Mb) is produced which has a red color like oxymyoglobin. Carboxymyoglobin exhibited greater resistance to oxidative degradation than oxymyoglobin, maintaining red color stability for up to 24 days. These results demonstrate that gamma irradiation at 5 - 10 kGy effectively enhances and stabilizes the redness of traditional pork sausage throughout 24 days of storage, highlighting its potential as a strategy for preserving color quality.

Table 4 Color parameters (L, a, b) of traditional pork sausage (Num-Tub) during storage at different irradiation doses.

Storage Time (day)	Irradiation doses (kGy)	Color parameters		
		L	a	b
0	Non-irradiation	46.52 ± 0.38 ^a	4.78 ± 0.41 ^b	10.99 ± 0.10 ^a
	5	43.17 ± 0.94 ^b	5.96 ± 0.11 ^a	10.24 ± 0.24 ^b
	10	43.37 ± 0.68 ^b	6.61 ± 0.40 ^a	10.36 ± 0.20 ^b
3	Non-irradiation	49.11 ± 1.08 ^a	4.81 ± 0.48 ^b	9.91 ± 0.21 ^b
	5	45.38 ± 2.00 ^b	6.10 ± 0.43 ^a	10.80 ± 0.43 ^a
	10	44.84 ± 1.05 ^b	6.09 ± 0.52 ^a	10.45 ± 0.47 ^{ab}
6	5	47.47 ± 1.06 ^a	6.63 ± 0.07 ^a	9.62 ± 0.20 ^b
	10	46.58 ± 1.27 ^a	6.38 ± 0.34 ^a	11.24 ± 0.14 ^a
9	5	47.44 ± 0.31 ^a	6.56 ± 0.20 ^a	9.17 ± 0.33 ^b
	10	45.81 ± 0.94 ^a	6.35 ± 0.23 ^b	10.42 ± 0.06 ^a
12	10	46.26 ± 1.70	6.40 ± 0.25	11.38 ± 0.41
15	10	44.67 ± 0.77	6.50 ± 0.38	10.29 ± 0.57
18	10	45.13 ± 0.72	6.40 ± 0.10	10.65 ± 0.06
21	10	45.28 ± 1.49	6.57 ± 0.41	10.34 ± 0.51
24	10	46.33 ± 0.51	6.36 ± 0.05	10.61 ± 0.29

Notes: Mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Correlation analysis of microbial, chemical, and color parameters

The Pearson correlation coefficients among microbial, chemical, and color parameters in Num-Tub pork sausage are presented in **Figure 3**. Pearson correlation coefficients (r) were calculated to evaluate the strength and direction of linear relationships between parameters. Values of r range from -1 (strong negative correlation) to +1 (strong positive correlation), while values near 0 indicate weak or no correlation.

Microbial and chemical quality: Total viable count (TVC) and lactic acid bacteria (LAB) were strongly positively correlated with acidity ($r = 0.88, p < 0.01$) and negatively correlated with pH ($r = -0.85, p < 0.01$), indicating that higher microbial loads were associated with increased acidity and lower pH. TVC and LAB were also positively correlated with TBA values ($r = 0.72, p < 0.05$), suggesting that elevated microbial activity may promote lipid oxidation.

Color-related parameters: Myoglobin (Mb) and oxymyoglobin (MbO₂) were highly positively correlated with lightness (L), redness (a), and yellowness (b) ($r = 0.85 - 0.96, p < 0.01$), and negatively correlated with metmyoglobin (MetMb) ($r = -0.94$ to $-0.98, p < 0.01$),

indicating that pigment oxidation led to color deterioration. MetMb was further positively correlated with TVC/LAB ($r = 0.75, p < 0.05$) and acidity ($r = 0.71, p < 0.05$), highlighting the link between microbial spoilage, acidification, and meat browning. Lightness (L) and redness (a) were strongly influenced by Mb and MbO₂ (L: $r = 0.85 - 0.88, a: r = 0.95 - 0.96$), whereas b values showed weaker correlations with other parameters ($r = 0.35 - 0.64$), suggesting that yellowness was less sensitive to microbial, chemical, or pigment changes.

Lipid oxidation: TBA values exhibited moderate negative correlations with Mb ($r = -0.48$) and MbO₂ ($r = -0.52$) and a positive correlation with MetMb ($r = 0.58, p < 0.05$), indicating that lipid oxidation was associated with pigment degradation and color loss.

pH relationships: pH was moderately positively correlated with Mb and color parameters ($r = 0.52 - 0.57, p < 0.05$), suggesting that less acidic conditions help maintain myoglobin stability and redness.

The microbial growth during storage influenced chemical properties and lipid oxidation, whereas myoglobin oxidation was the primary factor determining color changes in Num-Tub pork sausage.

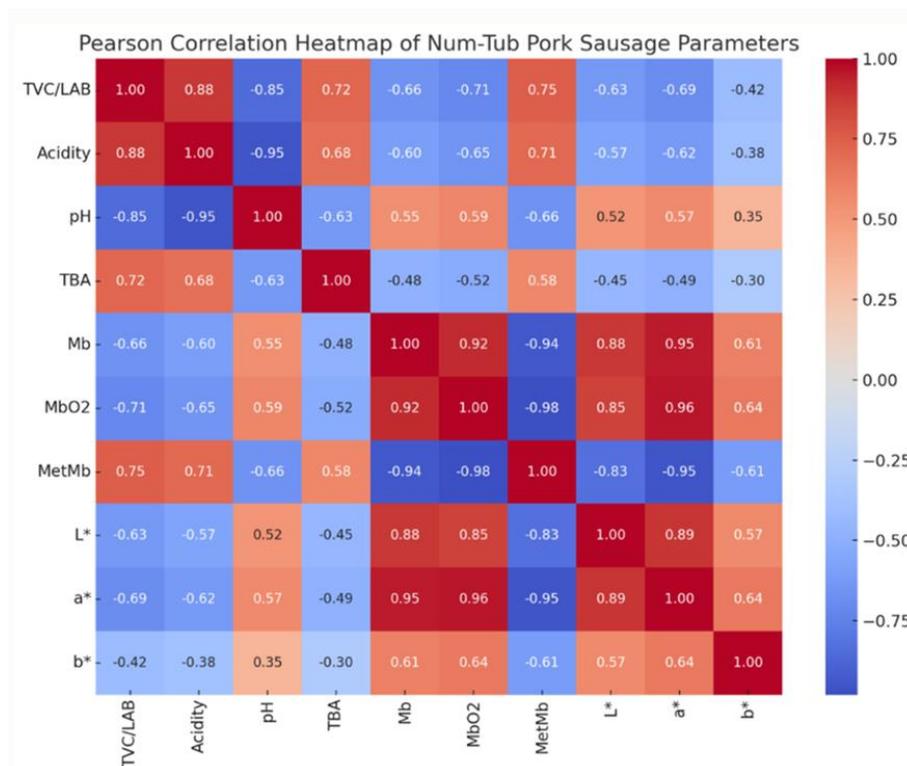


Figure 3 Heatmap showing Pearson correlation coefficients between microbial, chemical, and color parameters in Num-Tub pork sausage.

Conclusions

Gamma irradiation is a non-thermal food preservation method that does not alter the physical characteristics of Num-Tub. Irradiation at a dose of 10 kGy effectively improves microbial quality by reducing microbial load and controlling the regrowth of lactic acid bacteria up to 24 days at ambient storage. Regarding other quality attributes, such as color, oxymyoglobin content of 10 kGy irradiated pork Num-Tub peaked on day 9 of storage before gradually declining. This study concluded that gamma irradiation at a dose of 10 kGy can extend the shelf life of Num-tub at 28 ± 2 °C for up to 9 days. It enhances microbial safety, delays lactic acid formation, and promotes oxymyoglobin development, resulting in a redder appearance. However, it may concurrently stimulate lipid oxidation. For food safety policy, the evidence supports the adoption of irradiation as a complementary strategy to existing hygiene and cold-chain measures. This approach is particularly beneficial for high-risk ready-to-eat foods. From an industry perspective, standardized irradiation protocols could help small- and medium-scale producers deliver safer products with extended market reach while reducing reliance on chemical preservatives. However, further research is needed to identify optimal conditions for combining irradiation with natural antioxidants or modified packaging to reduce lipid oxidation. Moreover, assessing sensory quality alongside consumer acceptance is crucial for a comprehensive understanding.

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Declaration of generative AI in scientific writing

During the preparation of this manuscript, the authors utilized Microsoft Copilot and ChatGPT, AI-powered writing assistants, to improve readability and language. Following the application of this tool/service, the authors thoroughly reviewed and edited the content

as necessary and take full responsibility for the final published version.

CRedit author statement

Jaruratana Eamsiri: Conceptualization, Methodology, Investigation, Writing - Original Draft; **Ratchaneeporn Photinam:** Formal analysis, Investigation, Writing - Review & Editing; **Sirilak Chookaew:** Methodology, Investigation, Writing - Original Draft; **Khemruji Khemthong:** Methodology, Investigation; **Surasak Sajjabut:** Methodology, Investigation; **Wachiraporn Pewlong:** Project administration.

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