



## EFFECT OF GAMMA IRRADIATION ON MICROBIAL QUALITY AND PHYSICOCHEMICAL ATTRIBUTES OF TILAPIA (*Oreochromis niloticus*)

### EFEK IRADIASI GAMMA TERHADAP KUALITAS MIKROBA DAN FISIKO-KIMIA IKAN NILA (*Oreochromis niloticus*)

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#### Abstract

Tilapia (*Oreochromis niloticus*) is a widely consumed freshwater fish commodity that is highly susceptible to microbial spoilage, leading to rapid quality deterioration and a short shelf life. Gamma irradiation is a promising preservation technology to address this issue. This study aimed to evaluate the effectiveness of gamma irradiation in reducing microbial contamination and maintaining the physicochemical quality of fresh tilapia. Samples were irradiated at a dose of 3 kGy (dose rate 1 kGy/h) in combination with vacuum packaging and refrigeration. Microbiological analyses included total plate count, *Salmonella-Shigella*, and total mold and yeast, while physicochemical parameters comprised pH, ash, fat, protein, water activity (aw), total volatile base nitrogen (TVB-N), trimethylamine (TMA-N), and color (L\*, a\*, b\*), using non-irradiated samples as controls. The results showed that irradiation reduced total bacteria and *Salmonella-Shigella* by approximately 2 log cycles and eliminated molds and yeasts without significant changes ( $P > 0.05$ ) in physicochemical parameters compared to control samples. These findings indicate that gamma irradiation at 3 kGy is effective in improving microbial safety while preserving the physicochemical quality of fresh tilapia.

**Keywords:** Gamma Irradiation; Physicochemical; Tilapia (*Oreochromis niloticus*)

#### Abstrak

Ikan nila (*Oreochromis niloticus*) merupakan komoditas perikanan air tawar yang banyak dikonsumsi dan sangat rentan terhadap kerusakan akibat aktivitas mikroba, sehingga menyebabkan penurunan mutu dan umur simpan yang relatif singkat. Teknologi iradiasi gamma merupakan salah satu metode yang menjanjikan untuk mengatasi permasalahan tersebut. Penelitian ini bertujuan untuk mengevaluasi efektivitas iradiasi gamma dalam menurunkan kontaminasi mikroba serta mempertahankan kualitas fisikokimia ikan nila segar. Sampel diiradiasi pada dosis 3 kGy (laju dosis 1 kGy/jam) yang dikombinasikan dengan pengemasan vakum dan penyimpanan dingin. Analisis mikrobiologi meliputi total plate count, *Salmonella-Shigella*, serta total kapang dan khamir, sedangkan parameter fisikokimia meliputi pH, kadar abu, lemak, protein, aktivitas air (aw), total volatile base nitrogen (TVB-N), trimetilamina (TMA-N), serta warna (L\*, a\*, b\*), dengan sampel tanpa iradiasi sebagai kontrol. Hasil penelitian menunjukkan bahwa iradiasi mampu menurunkan total bakteri dan *Salmonella-Shigella* sebesar sekitar 2 siklus log serta mengeliminasi kapang dan khamir tanpa perubahan signifikan ( $P > 0,05$ ) pada parameter fisikokimia dibandingkan dengan sampel kontrol. Temuan ini menunjukkan bahwa iradiasi gamma pada dosis 3 kGy efektif dalam meningkatkan keamanan mikrobiologis sekaligus mempertahankan kualitas fisikokimia ikan nila segar.

**Kata Kunci:** Fisikokimia; Ikan nila (*Oreochromis niloticus*); Iradiasi gamma

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## INTRODUCTION

The freshwater aquaculture sector is one of the industries that is predicted to be a driver of national development and plays an important role in welfare, job creation, and the sustainability of natural resources (Oktopura et al., 2020). One type of freshwater fish that is popular in Indonesia is tilapia. Tilapia (*Oreochromis niloticus*) is a major commodity in the Indonesian fisheries industry, and is widely distributed throughout the country. Tilapia has become a leading commodity along with increasing production levels. Optimizing tilapia cultivation is essential to enhance production efficiency and support the increasing national demand, thereby contributing to the growth of total tilapia production at the national level (Lusiana et al., 2021). Tilapia is an important aquaculture commodity and a rich source of nutrients, particularly high-quality protein ( $\approx 18.46$  g/100 g), essential amino acids (e.g., lysine, leucine, arginine), and fatty acids. Its lipid fraction consists of saturated, monounsaturated, and polyunsaturated fatty acids, including linoleic acid, which contribute to metabolic functions and overall health (Nuryanto et al., 2022). This makes tilapia a promising complementary food for consumption by the Indonesian people.

Based on data from the Badan Pusat Statistik (BPS) (2025), tilapia production in 2023 reached 1.37 tons, exceeding production in 2021 by 1.17 tons. This production amount is the highest among other aquaculture products such as patin, catfish, and carp. This potential can make tilapia a food source for national consumption and a foreign exchange earner for the country through exports.

The main problem with fishery products is their susceptibility to damage and short shelf life. Therefore, proper handling and storage are crucial to slow down their deterioration. This is primarily due to fish products having a high water and protein content, which accelerates enzymatic and chemical reactions, leading to a loss of freshness and microbial decay. Furthermore, bacterial contamination in their habitat can also contribute to this spoilage (Irawati et al., 2019).

After fish die, the activity of spoilage bacteria increases, leading to an accelerated reduction of Trimethylamine Oxide (TMAO) to Trimethylamine (TMA). Total Volatile Base Nitrogen (TVB-N) is also formed through microbial activity following the breakdown of proteins and non-protein nitrogen compounds. TVB-N represents a group of biogenic amines, including trimethylamine, dimethylamine, and ammonia. Consequently, TVB-N is a key parameter used to evaluate the freshness and shelf life of fish products (Herman & Abdel-naeem, 2023). Furthermore, *Salmonella*, *E. coli*, and *S. aureus* are among the most common pathogenic bacteria found in fish and fish products that cause foodborne illnesses (Mahmoud et al., 2022).

Chilling is the most widely used method for preserving fish, whether intended for fresh consumption or as a pre-treatment before freezing. However, this method leads to a decline in fish quality during storage, particularly affecting texture, color, and taste, thereby reducing its shelf life (Tavares et al., 2021). The combination of gamma irradiation technology with chilling can be a solution to challenges concerning fish products, including tilapia. Gamma irradiation is a non-thermal (cold) process, meaning there is no temperature increase during the process, thus maintaining the quality of fish products (Mohamed et al., 2023). The right dose of irradiation is needed to extend the shelf life without reducing the physical, chemical, and sensory quality of food products. An irradiation dose of 3 kGy can reduce the total microbes by 3 log cycles in jenuha fish fillets and 2 log cycles in rainbow trout (Moslemi & Naderi, 2022; Pangestika et al., 2022). El-Ghafour and Zakar (2017) reported that 3 kGy was the best irradiation dose to reduce total microbes and extend the shelf life of tilapia fillets.

This study aims to determine the effect of gamma irradiation on the elimination of microorganisms in fresh tilapia and its impact on the fish's physicochemical characteristics. Researchers expect these findings to provide a basis for utilizing irradiation technology to eliminate microorganisms and, consequently, extend the shelf life of fresh freshwater fish.

## MATERIALS AND METHODS

### Research Design

This study employed a quantitative experimental approach using a completely randomized design (CRD). Fresh tilapia (*Oreochromis niloticus*), weighing approximately 250 g per sample ( $\approx 23$

× 9 cm), were purchased alive from a local fish market in Parung, Bogor, and euthanized on-site. The samples were immediately transported to the laboratory in a cooled container to minimize postmortem deterioration, then cleaned and vacuum-packaged using nylon–polyethylene (PE) film.

The vacuum-packaged samples were randomly assigned to two treatment groups: non-irradiated (0 kGy, control; n= 5) and irradiated (3 kGy; n= 5), then stored at –20 °C until analysis. Non-irradiated samples underwent identical handling without irradiation. Microbiological and physicochemical analyses included total plate count (TPC), *Salmonella-Shigella*, total mold and yeast, pH, ash, fat, protein, water activity ( $a_w$ ), total volatile base nitrogen (TVB-N), trimethylamine (TMA-N), and color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ).

### Gamma Irradiation

Gamma irradiation was performed using a Cobalt-60 gamma irradiator (IRKA) at the irradiation facility of KST G.A. Siwabessy, BRIN, Lebak Bulus, Indonesia. Vacuum-packaged samples were irradiated at an average dose of 3 kGy with a dose rate of 1 kGy/h.

The selected dose of 3 kGy falls within the medium-dose range (1–10 kGy) specified by SNI 8275:2016 and was chosen based on its effectiveness in reducing spoilage and pathogenic microorganisms while preserving physicochemical quality. Previous studies have shown that irradiation doses of 2–5 kGy can achieve microbial reductions of approximately 1–3 log cycles and extend the shelf life of fresh fish without significant changes in pH, lipid oxidation, protein content, or sensory attributes (Al-Kuraieef, 2021). Therefore, a dose of 3 kGy was considered optimal for balancing microbial safety and quality retention in fresh tilapia (*Oreochromis niloticus*).

### Microbiological Analysis

Total Plate Count (TPC) and mold-yeast analyses were conducted using the serial dilution method. TPC was determined following the method by Said et al. (2023) utilizing Plate Count Agar (PCA). Mold and yeast counts were determined following the method by Saraswathi et al. (2023) utilizing Sabouraud Dextrose Agar (SDA). *Salmonella* and *Shigella* analysis was performed using a swab technique based on the method by Lazaro et al. (2020), utilizing *Salmonella-Shigella* agar. All results are expressed as CFU/g.

### Physico-Chemical Analysis

Water activity ( $a_w$ ) was measured using an HD 3A  $a_w$  meter. pH was determined after dissolving 3 g of tilapia in 30 mL of water, using a Mettler Toledo Seven Compact S220 pH/Ion meter. Water content and ash content were determined using the gravimetric method, according to SNI 2354.2:2015 and SNI 2354.1:2010, respectively. Fat content was determined using the Soxhlet extraction method, based on SNI 2354.3-2017. Protein content was determined using the Kjeldahl method, following SNI 01-2354.4-2006. Total carbohydrate content was calculated by the difference method, based on (Kakatkar et al., 2024), with the following calculations, %carbohydrate= 100% - (moisture content + ash content + fat content + protein content).

Color determination is measured using the Nix Color Sensor from Canada. The color scanning process utilizes Bluetooth, with the Nix Color Sensor application (Nix QC app) previously installed on a mobile phone. Calibration is performed by scanning the barcode on the reference tile. Subsequently, measurements are taken by placing the Nix Color Sensor on the flat surface of the tilapia sample. The purpose of this color scanning is to obtain the  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) values (Swetha & Chakraborty, 2021). Color measurement uses the *CIELab* reference, where L indicates brightness on a scale of 0 to 100 (from black to white);  $a^*$  indicates red when the value is positive and green when negative; and  $b^*$  indicates yellow when positive and blue when negative (Xie et al., 2023). TVB-N and TMA-N analysis used the titration method based on the Indonesian National Standard (SNI 2354.8, 2009) for the determination of Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) levels in fishery products.

## Data Analysis

The analysis data were processed using Microsoft Office Excel Home and Student 2019. Statistical analysis was conducted using an independent t-test in IBM SPSS software version 27.

## RESULTS

The results of the analysis for microbiology, pH,  $a_w$ , proximate composition, color, Total Volatile Base Nitrogen (TVB-N), and Trimethylamine Nitrogen (TMA-N) of tilapia samples are presented in the table below. Based on the microbiological analysis results presented in Table 1, the Total Plate Count (TPC) and *Salmonella-Shigella* showed a 2-log cycle decrease in bacterial numbers after gamma irradiation treatment, and molds and yeasts were eliminated in tilapia products. The TPC values for both control and 3 kGy gamma-irradiated tilapia met the requirements of SNI 2729:2013 concerning fresh fish, which specifies a maximum of  $5.0 \times 10^5$  CFU/g.

**Table 1.** Effects of gamma irradiation on microbiological parameters of tilapia

Parameter	Irradiation dose		SNI 2729:2013 fresh fish (CFU/g)
	0 kGy (CFU/g)	3 kGy (CFU/g)	
Total Plate Count	$3.23 \times 10^5$	$2.78 \times 10^3$	$< 5.0 \times 10^5$
Mold-yeast	$2.28 \times 10^3$	n.d.	-
<i>Salmonella-Shigella</i>	$4.32 \times 10^3$	$3.33 \times 10^1$	<i>Salmonella</i> value: negatif

Note: n.d= not detected

Based on the pH value measurements of tilapia samples irradiated with gamma radiation at a dose of 3 kGy, no significant difference was observed when compared to the control (0 kGy). The obtained values were based on three measurements for each sample (Table 2). For the water activity ( $a_w$ ) value, a significant difference ( $P < 0.05$ ) was found between the control tilapia samples and those exposed to 3 kGy gamma radiation.

**Table 2.** Effect of gamma irradiation on physicochemical values of tilapia

Parameter	Irradiation dose	
	0 kGy	3 kGy
pH	$7.09 \pm 0.12^a$	$6.99 \pm 0.08^a$
$a_w$	$0.862 \pm 0.02^a$	$0.907 \pm 0.003^b$
Water content (% wb)	$78.71 \pm 0.37^a$	$76.97 \pm 1.26^a$
Ash content (% wb)	$0.983 \pm 0.24^a$	$1.097 \pm 0.13^a$
Protein content (% wb)	$11.10 \pm 0.19^a$	$11.57 \pm 0.13^b$
Fat content (% wb)	$0.32 \pm 0.01^a$	$0.28 \pm 0.07^a$
Carbohydrate content (% wb)	8.89	10.08
TVB-N (mg N/100g)	$10.00 \pm 0.74^a$	$11.13 \pm 0.48^a$
TMA-N (mg N/100g)	$1.05 \pm 1.49^a$	$0.00 \pm 0.00^a$

Note: Numbers in the same row followed by different letters indicate significant differences ( $P < 0.05$ ) using the independent t-test

Based on the data in Table 2, gamma irradiation at a dose of 3 kGy slightly increased the ash content of tilapia, from 0.983% in control samples to 1.097% in irradiated samples. Similarly, protein content increased from 11.10% (control) to 11.57%, and carbohydrate content rose from 8.89% (control) to 10.08%. Conversely, a slight decrease due to 3 kGy gamma irradiation was observed in both water and fat content. Water content decreased from 78.71% (control) to 76.97%, while fat content saw a reduction from 0.32% (control) to 0.28% in the 3 kGy irradiation dose treatment.

The level of fish freshness is determined by the values of Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N). Based on the measurement results in Table 2, the TVB-N value for the 3 kGy gamma irradiation dose treatment was 11.13 mg N/100g, which was slightly higher than the control value of 10.00 mg N/100g. However, this difference was not statistically significant. The TMA-N measurement results showed that TMA-N was not detected in tilapia samples treated with gamma irradiation, indicating that the level of fish freshness was still well maintained.

Based on the data in Table 3, the L\* value (brightness) in the control sample is slightly higher than that of the sample with 3 kGy irradiation treatment. Meanwhile, the a\* value (redness) shows no significant difference between the irradiated and control treatments, remaining stable with values indicating a greenish hue. For the b\* value (yellowness), both treatments indicate a yellowish hue, and the control value is higher than that of the irradiated sample. Statistically, for the L\*, a\*, and b\* values, there is no significant difference between the control and irradiated samples.

**Table 3.** Effects of gamma irradiation on the color value of tilapia

Irradiation dose	Color value		
	L*	a*	b*
0 kGy	47.47 ± 3.00 <sup>a</sup>	-2.43 ± 3.39 <sup>a</sup>	1.28 ± 1.42 <sup>a</sup>
3 kGy	46.38 ± 3.23 <sup>a</sup>	-2.43 ± 2.12 <sup>a</sup>	0.88 ± 1.92 <sup>a</sup>

Note: Numbers in the same column followed by different letters indicate significant differences ( $P < 0.05$ ) using the independent t-test

## DISCUSSION

Fresh fish products are highly susceptible to external contamination, which can lead to foodborne diseases. This contamination is primarily associated with pathogenic microorganisms, including bacteria, fungi, parasites, and viruses. Total Plate Count (TPC) serves as an indicator of the overall level of microbial contamination in food (Mohamed et al., 2023). Microbial growth is the principal cause of fish spoilage and significantly affects the quality of fresh fish. After death, fish are rapidly colonized by bacteria originating from the skin and the surrounding environment. In addition, intrinsic factors such as high water activity, near-neutral pH (>6), and the presence of non-protein nitrogen compounds promote rapid microbial proliferation, resulting in undesirable changes in appearance, texture, odor, and taste (Tavares et al., 2021).

In this study, gamma irradiation at a dose of 3 kGy effectively reduced the TPC from  $3.23 \times 10^5$  CFU/g in the control sample to  $2.78 \times 10^3$  CFU/g, corresponding to a reduction of approximately two log cycles. This result is consistent with previous findings indicating that medium-dose irradiation (2–5 kGy) can significantly reduce microbial populations in fish products (Moslemi & Naderi, 2022). Furthermore, the observed TPC values remained below the maximum permissible limit of  $5.0 \times 10^5$  CFU/g established by SNI 2729:2013, confirming that the treated samples met microbiological safety standards. In addition to bacterial reduction, irradiation at 3 kGy was also effective in eliminating molds and yeasts in tilapia samples.

The presence of pathogenic bacteria such as *Salmonella-Shigella* in fish products remains a major food safety concern. Wali et al. (2020) reported the presence of *Salmonella-Shigella* in several seafood products, indicating potential contamination risks associated with handling practices and environmental exposure. According to food safety standards, *Salmonella* must be absent in fishery products intended for consumption due to its potential to cause gastrointestinal infections. Therefore, irradiation treatment plays an important role in improving microbial safety, particularly in reducing pathogenic bacteria in fresh fish.

The effectiveness of gamma irradiation in microbial inactivation is closely related to radiation-induced chemical reactions, particularly the radiolysis of water. Ionizing radiation interacts with water molecules to produce reactive species such as hydroxyl radicals ( $\bullet\text{OH}$ ), hydrated electrons ( $e^-_{aq}$ ), and hydrogen atoms ( $\bullet\text{H}$ ). Among these, hydroxyl radicals are highly reactive and play a dominant role in damaging cellular components. These reactive species induce oxidative damage to microbial DNA, leading to single- and double-strand breaks. While single-strand breaks may be repaired by cellular mechanisms, the accumulation of damage and the formation of double-strand breaks result in irreversible DNA damage and ultimately cell death. This mechanism explains the significant reduction of microbial populations observed in irradiated samples (Raj et al., 2025; Ravindran & Jaiswal, 2019)

Based on the data in Table 2, no significant changes were observed in the pH values of either control or irradiated samples. This finding is consistent with previous studies reporting that gamma irradiation at moderate doses does not significantly affect the pH of fish products (Uikey et al., 2024;

Xiao et al., 2025). The relatively stable pH indicates that irradiation at 3 kGy did not induce substantial chemical degradation of nitrogenous compounds in fish muscle (Xie et al., 2023). Changes in pH in fish are generally associated with postmortem biochemical processes, particularly the accumulation of lactic acid due to glycolysis (Perceka et al., 2020). In contrast, a slight but statistically significant increase in water activity ( $a_w$ ) was observed in irradiated samples (from 0.862 to 0.907). This increase may be attributed to structural modifications in the muscle matrix induced by irradiation. Ionizing radiation can disrupt non-covalent interactions within muscle proteins and weaken the integrity of muscle fibers, leading to a softer tissue structure and increased availability of free water. Similar observations have been reported in shrimp, where irradiation at 3 kGy resulted in muscle softening (Wang et al., 2023). These findings indicate that gamma irradiation at 3 kGy primarily affects the physical structure of muscle tissue rather than causing significant chemical degradation. Therefore, the absence of significant pH changes does not contradict the observed increase in water activity, as both parameters reflect different aspects of quality: pH represents chemical stability, whereas water activity is more closely related to structural changes and water mobility within the tissue. Although higher water activity may increase susceptibility to microbial growth, this effect can be mitigated by appropriate storage conditions, such as freezing or refrigeration, which were applied in this study (Xie et al., 2023).

Previous research by Mohamed et al. (2023) indicated that gamma irradiation treatment did not have a significant effect on the proximate composition of tilapia. The results showed no significant differences in moisture, ash, and fat content between control and irradiated samples. A slight decrease in moisture content may be attributed to the loss of free water, while minor lipid degradation may occur due to irradiation-induced oxidative reactions (Raj et al., 2025). The observed increase in ash content is likely a relative effect resulting from reductions in other components, leading to a proportional increase in mineral content. Although a statistically significant difference in protein content was observed, the overall value remained relatively stable at approximately 11%, indicating minimal impact on protein integrity.

A slight increase in TVB-N values was observed following irradiation, which may be attributed to protein degradation and increased proteolytic activity (Dalle et al., 2021). However, this increase was not statistically significant and remained within acceptable limits ( $<20$  mg N/100 g) for fresh fish products (Perceka et al., 2020). These findings are consistent with previous studies reporting no significant differences between irradiated and control samples (El-Ghafour & Zakar, 2017). In contrast, TMA-N values in irradiated samples were lower than those in control samples, indicating reduced formation of trimethylamine. TMA-N is an important freshness indicator that can accumulate in fish muscle during storage, particularly under chilled conditions. El-Ghafour and Zakar (2017) reported that tilapia fillets treated with 3 kGy irradiation exhibited lower TMA-N values (4.9 mg N/100 g) compared to control samples (5.5 mg N/100 g), which is consistent with the results of this study. The relatively low TMA-N levels in tilapia may also be attributed to the low trimethylamine oxide content in its muscle tissue (Monteiro et al., 2018).

Color is a critical parameter influencing consumer acceptance of fish products (Herman & Abdel-naeem, 2023). The results showed no significant differences in color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) between control and irradiated samples, indicating that gamma irradiation at 3 kGy did not adversely affect visual quality. In addition to maintaining color, irradiation has been reported to preserve sensory attributes such as taste and odor without leaving radioactive residues, supporting its application as a safe preservation technology (Mohamed et al., 2023).

## CONCLUSION

Based on the results of this study, gamma irradiation at a dose of 3 kGy effectively reduced total bacteria and *Salmonella-Shigella* by approximately 2 log cycles and eliminated molds and yeasts in tilapia. The treatment did not cause significant changes in physicochemical characteristics and maintained product freshness, as indicated by TVB-N values below 20 mg N/100 g. These findings demonstrate that gamma irradiation at 3 kGy is an effective method for improving microbial safety while preserving the physicochemical quality and freshness of tilapia (*Oreochromis niloticus*).

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