

# IMPROVEMENT OF THE SHELF LIFE AND NUTRITIONAL QUALITY OF FERMENTED MUSHROOMS (PEKASAM CENDAWAN) THROUGH GAMMA RADIATION AND OPTIMIZED STORAGE CONDITIONS

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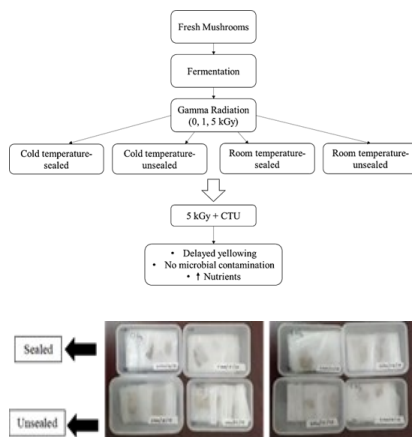
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## Graphical abstract



## Abstract

Fresh mushrooms typically have a shelf life of about three days before environmental factors begin to affect their quality. To extend, various processing methods have been explored, including drying and canning. In this study, fermentation combined with gamma radiation (0, 1, and 5 kGy) was explored to increase shelf life. Hence, to evaluate the characteristics of fermented mushrooms (pekasam cendawan) over a 12-day storage period under different conditions, were used; cold temperature-sealed (CT-S), cold temperature-unsealed (CT-U), room temperature-sealed (RT-S), and room temperature-unsealed (RT-U). Key parameters assessed included color, texture, and microbial contamination. The results showed that by days 9 and 12, yellow spots appeared on non-irradiated samples stored in CT-S packaging, while those in CT-U packaging exhibited yellowing by day 12. Meanwhile, radiation at 1 kGy delayed yellowing in some samples, whereas 5 kGy caused yellowing in CT-S samples by day 12. Radiation significantly influenced color, particularly in the 1 and 5 kGy samples compared to non-irradiated ones. Texture also exhibited significant changes ( $p < 0.05$ ) across various conditions and radiation doses. Notably, microbial contamination was absent in both irradiated (1 and 5 kGy) and non-irradiated samples. Further analysis of mushrooms irradiated at 5 kGy and stored under CT-U conditions revealed higher ash (2.5%), protein (4.1%), and carbohydrate (12.7%) content compared to non-irradiated controls (2.1%, 3.3%, and 11.4%, respectively). Moisture content was slightly lower in irradiated samples (80.3% vs. 82.8%), while fat (0.4%) and fiber (<0.1%) remained unchanged. In conclusion, gamma radiation improved the preservation and nutritional quality of fermented mushrooms with minimal adverse effects on their physical and chemical properties.

**Keywords:** Gamma radiation, fermented mushrooms, shelf-life extension, storage conditions, nutritional quality

## Abstrak

Cendawan segar biasanya mempunyai jangka hayat sekitar tiga hari sebelum terjejas oleh faktor persekitaran. Untuk memanjangkan jangka hayatnya, pelbagai kaedah pemprosesan telah diterokai, termasuk pengeringan, pengefinan, dan Dalam kajian ini, kaedah di radiasi sinaran gamma (0, 1, dan 5 kGy). Kajian ini bertujuan untuk menilai ciri-ciri pekasam cendawan sepanjang tempoh penyimpanan 12 hari di bawah pelbagai keadaan: suhu sejuk-pateri (CT-S), suhu sejuk-tanpa pateri (CT-U), suhu bilik-pateri (RT-S), dan suhu bilik-tanpa pateri (RT-U). Ciri-ciri utama yang dinilai termasuk warna, tekstur, dan pencemaran mikrob. Tompok kuning muncul pada sampel tanpa radiasi yang disimpan dalam bungkusan CT-S pada hari ke-9 dan 12, serta dalam bungkusan CT-U pada hari ke-12. Radiasi pada 1 kGy melambatkan kekuningan pada beberapa sampel, manakala 5 kGy menyebabkan kekuningan dalam sampel CT-S pada hari ke-12. Radiasi memberi kesan ketara terhadap warna ( $p < 0.05$ ), terutamanya pada sampel 1 dan 5 kGy berbanding sampel tanpa radiasi. Tekstur juga menunjukkan perubahan ketara ( $p < 0.05$ ) dalam keadaan tertentu merentasi semua dos radiasi. Pencemaran mikrob tidak dikesan dalam semua sampel yang diberi perlakuan radiasi (1 dan 5 kGy) serta sampel tanpa radiasi. Analisis lanjut terhadap cendawan yang diradiasi 5 kGy yang disimpan di bawah CT-U menunjukkan kandungan abu (2.5%), protein (4.1%), dan karbohidrat (12.7%) yang lebih tinggi berbanding sampel tanpa radiasi (2.1%, 3.3%, 11.4%). Kandungan lemak lebih rendah sedikit dalam sampel diradiasi (80.3% berbanding 82.8%), manakala lemak (0.4%) dan serat (<0.1%) kekal tidak berubah. Kesimpulannya, radiasi akut meningkatkan pemeliharaan dan kualiti nutrisi cendawan pekasam, menghasilkan kesan buruk yang minimum terhadap ciri fizikal dan kimia.

**Kata kunci:** Radiasi gamma, cendawan pekasam, pemanjangan jangka hayat, keadaan penyimpanan, kualiti nutrisi

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## 1.0 INTRODUCTION

Nutrient and iron deficiencies remain a significant global concern, affecting more than 80% of the human population [1]. According to the Food and Agriculture Organization, over 925 million people suffered from nutrient deficiencies in 2009, and between 1993 and 2005, more than 30% of the population experienced anemia due to inadequate nutrition (FAO, 2012) [2]. Hence, incorporating mushrooms into daily diets can be beneficial for individuals with underlying health conditions due to its rich nutritional content [3]. Mushrooms are also an excellent source of niacin, containing levels more than 10 times higher than those found in vegetables [4]. Additionally, they thrive in shaded, moist environments with suitable temperatures, making them a sustainable and accessible food source [5].

In recent years, mushrooms have been commercialized not only in their fresh form but also as innovative products such as fermented mushrooms. These are produced through controlled fermentation methods, enhancing their flavor, texture, and shelf life. Fermented foods have long been a staple in various cultural diets and are now gaining popularity in Western countries due to their health benefits, particularly for the digestive system [6]. To maximize these benefits, proper fermentation techniques must be implemented. Additionally, fermented foods

promote digestive health while extending shelf life, reducing the risk of spoilage.

Among these, mushrooms from *Pleurotus* sp. have recently garnered the attention of researchers due to their myriad bioactive compounds, which confer several health benefits. *Pleurotus*, commonly known as oyster mushrooms, represents a genus of edible fungi [7]. One of the primary reasons oyster mushrooms have attracted interest is their rich content of essential nutrients, including proteins, dietary fibers, vitamins, and essential minerals, which support a healthy diet and contribute to overall wellness [8]. Additionally, advancements in cultivation technology have enabled oyster mushrooms to grow not only on wooden logs but also in transparent polystyrene bags and mushroom blocks. They thrive in shaded environments with high humidity and temperatures suitable for tropical and subtropical climates [9].

Gamma radiation, a high energy form of electromagnetic radiation, is increasingly used in food treatment processes due to its efficacy in reducing microbial loads, prolonging shelf life, and ensuring food safety. Regulatory authorities such as the FDA endorse the use of gamma radiation, particularly emphasizing doses around 1-10 kGy to effectively eliminate pathogens while maintaining the nutritional quality of food products [10]. Research indicates that while low doses generally do

not induce significant changes in nutritional or organoleptic properties, higher doses can lead to oxidative degradation and nutritional losses, especially of sensitive compounds like vitamins and phytochemicals [11]. Specific studies affirm that gamma radiation can effectively degrade mycotoxins and pesticidal residues in food products, which poses a dual benefit of microbial inactivation and toxin reduction [12,13].

Comparatively, other forms of electromagnetic radiation like X-rays and UV radiation, though effective, possess different penetration capabilities and biological interactions. X-rays, while also ionizing, typically require more controlled doses due to higher energy levels that can significantly alter molecular structure and thus entail greater regulatory scrutiny in food applications [14]. UV radiation, on the other hand, primarily impacts surface microorganisms and may not deeply penetrate food matrices, offering less efficacy in terms of thorough disinfection and preservation for perishable items [15,16].

Gamma irradiation has been applied in food systems including mushrooms, but there is limited scientific literature addressing its effects specifically on fermented mushroom products such as *pekasam cendawan*, especially in relation to storage duration, nutritional quality, and microbial safety under varying packaging and temperature conditions. Existing studies tend to focus either on fresh mushrooms or generic fermented foods, with minimal emphasis on the interactive effects of radiation and post-processing storage strategies.

In Malaysia, oyster mushrooms are widely commercialized in transparent polypropylene bags. During cultivation, they undergo several maturation stages on racks, requiring ample space for successful growth. After harvesting, these mushrooms can also be processed into fermented products [17]. Research has been conducted on the effects of gamma irradiation on fermented mushrooms to assess its impact on shelf life [18]. Therefore, this study specifically aimed to evaluate the durability and storage longevity of fermented mushrooms exposed to different levels of acute gamma radiation under various packaging and storage conditions. Additionally, this study examined the physical changes in irradiated mushrooms, focusing on color, texture, and contamination under different temperatures and packaging conditions. Finally, a proximate analysis was conducted to determine the nutritional content of the fermented mushrooms. Hence, this study aimed to evaluate the effects of gamma radiation (0, 1, and 5 kGy) and different storage conditions on the shelf life, physical quality (color and texture), microbial safety, and nutritional composition of fermented mushrooms (*Pekasam Cendawan*) over a 12-day period.

## 2.0 METHODOLOGY

### Study Material Source

Commercially produced fermented mushroom samples were obtained from the Malaysian Nuclear Agency (Nuklear Malaysia) in Dengkil, Selangor, with preparation conducted by research officers, as illustrated in Figure 1. The fermented mushroom samples were prepared under both control and irradiated conditions, with radiation doses of 1 kGy and 5 kGy. The control sample was the one without any radiation dose. All raw materials were provided by the agency for the preparation of the fermented mushrooms.



**Figure 1** Commercially available fermented mushroom samples were separated and packaged according to radiation doses of 0, 1, and 5 kGy

### Radiation Source

The radiation procedure was carried out at the radiation laboratory of the Malaysian Nuclear Agency (Nuklear Malaysia) in Dengkil, Malaysia. Fermented mushroom samples were exposed to gamma radiation at doses of 1 kGy and 5 kGy using a Cobalt-60 source in a designated decontamination room [19].

### Pre-Packaging Preparation of Fermented Mushroom

The fermented mushroom samples were prepared by the Malaysian Nuclear Agency (Nuklear Malaysia) in Dengkil, Malaysia. The packaging process involved both sealed and unsealed plastic packaging methods, as shown in Figure 2. Each package contained approximately 5 g of fermented mushrooms and was prepared using either a heat sealer (for sealed packaging) or a stapler (for unsealed packaging). In this context, sealed packaging prevents air exchange between the interior and exterior of the package, while unsealed packaging allows airflow. For this experiment, both airtight and regular plastic packaging were used. A total of 180 packages were prepared for each radiation dose group (0, 1, and 5 kGy), with each group consisting of 30 samples per treatment across four storage conditions [20].



**Figure 2** Mushroom packaging in sealed (S) and unsealed (US) forms

Before packaging, the fermented mushrooms were cut into small pieces of approximately 1 cm. They were then packaged using both sealed and unsealed methods, with 30 small packages allocated for each radiation dose (0, 1, and 5 kGy) for over five days, following a Full Factorial experimental design. The 30 packages were further divided into two storage conditions: 15 packages (both sealed and unsealed) were stored at refrigerated temperatures (8°C), while the remaining 15 were stored at room temperature (28°C to 30.2°C) [20].

### Gamma Radiation of Fermented Mushroom

The mushrooms were exposed to different doses of gamma radiation: 0 kGy (control), 1 kGy, and 5 kGy. The radiation dose is critical in determining the quality and shelf life of irradiated food. To achieve optimal preservation, it is essential to find the right balance. Insufficient radiation may fail to prevent spoilage, while excessive doses can negatively impact food quality [21]. Therefore, identifying the appropriate radiation dose is crucial to avoiding both under- and over-irradiation. According to the Food Act 1983 and the Food Irradiation Regulations 2011, the maximum permissible radiation dose for food is 10 kGy [22].

### Storage of Fermented Mushrooms

After radiation, fermented mushrooms were stored at two different temperatures: approximately 8°C in a refrigerator and between 28°C to 30.2°C at room temperature in the laboratory. These temperatures were monitored and recorded throughout the experiment, ensuring consistency within the specified ranges. Typically, cold storage is maintained at around 4°C; however, in this study, the refrigerator temperature was closer to 8°C, likely due to the presence of other food items. The relatively high room temperature, ranging from 28°C to 30.2°C, may have resulted from fluctuating weather conditions. Temperature measurements were verified using a Pigeon® digital thermometer (China). Proper storage was essential to maintaining the quality and extending the shelf life of the fermented mushrooms.

Sealed packages of fermented mushrooms irradiated at 0, 1, and 5 kGy were stored in a refrigerator at approximately 8°C. A total of 90 samples were stored for 12 days, with observations recorded on day 0 and every 3 days until day 12. Cold storage effectively slowed fungal growth,

whereas room temperature was found to promote fungal growth if the mushrooms were left for extended periods. Fermentation techniques were applied to extend shelf life. Physical changes including color, texture, and contamination were documented every 3 days [23]. For unsealed packages of fermented mushrooms irradiated at 0, 1, and 5 kGy, storage was conducted at room temperature, ranging from 28.0°C to 30.2°C. Similarly, 90 samples were observed following the same schedule. Temperature was continuously monitored using a digital thermometer throughout the storage period [24].

### Quality Control Evaluation

The fermented mushroom samples were stored under refrigerated conditions (8°C) and at room temperature (28°C to 30.2°C) to observe physical changes over time. Visual changes were monitored and recorded every three days, from day 0 to day 12, for both storage conditions. Observations focused on variations in color, texture, and contamination. Several key parameters such as weight, color, texture, and pH were analyzed to assess the effects of radiation on the physicochemical properties of different mushroom types. Figure 3 provides a visual representation of the physical changes in the fermented mushrooms over time.



**Figure 3** Quality control visualization and assessment of the fermented mushrooms via: a) colour; b) textural & c) contamination and fungal growth

In this study, the color of the fermented mushrooms was visually observed, and all images were captured using a digital microscope and analyzed with an image analysis application (Image Analyzer: Softonic version 1.42.1). The CIELAB color system (L, a, b) was used as the objective indicator to measure color changes in fermented mushrooms. This color space provides quantitative evaluation of lightness (L), red-green (a), and blue-yellow (b) components. Color changes were documented using a portable digital microscope (RS PRO USB Digital Microscope, 2M pixels, 20 to 200X magnification; Shanghai, China) values were recorded at regular intervals. The captured images

were then converted into grayscale profiles, where each pixel was assigned a single intensity value ranging from 0 (black) to 255 (white). Data and images were recorded from both the top and bottom petals of the fermented mushrooms [25].

The texture of the mushrooms was evaluated manually through visual inspection. Additionally, an image of the central area was captured using a microscope, focusing on the mushroom's core to observe its internal texture at a microscopic level. Texture was assessed manually by determining whether the mushroom was hard or watery and rated on a two-point scale, where 1 indicated "non-watery" and 2 indicated "watery." Contamination was monitored through visual inspection, with each sample examined for signs of spore formation or bacterial spots on the surface. Particular attention was given to black or green discoloration on the fermented mushrooms. In cases of contamination, an image of the affected sample was recorded [26].

#### Preparation of Control (Non-Radiated) and 5 kGy CT-U Radiated Samples

A 100 g sample of commercial fermented mushrooms was prepared for both non-radiated and 5 kGy irradiated samples. The ingredients used included *Pleurotus pulmonarius* mushrooms, 1% (w/w) of rice, and 2% (w/w) of salt. The preparation process involved cleaning the mushrooms and marinating them with salt. The rice was roasted until golden brown, then ground into a fine powder. The ground rice was mixed with the mushrooms and left to ferment for 1 to 2 days. After fermentation, the mushrooms were irradiated and stored in cold storage for 12 days.

The proximate analysis was conducted at UNIQE (M) Sdn. Bhd, Bangi, to determine the optimal storage duration for irradiated fermented mushrooms. The fermented mushroom and control samples (without gamma radiation) underwent moisture, ash, protein, crude fiber, and fat content analysis in accordance with the Association of Official Analytical Chemists (AOAC), 2010 [27] standard method. Meanwhile, the carbohydrate content in non-fermented and lacto-fermented mushrooms was determined using the Promerance Food Analysis methodology [28].

#### Ash Content in Fermented Mushrooms

The ash content was determined through combustion in a muffle furnace for approximately three hours, following the AOAC (2010) standard method [27]. The ash content was then calculated using the following formula:

$$\text{Ash Content (g/100 g sample)} = (\text{Weight of Ash} / \text{Weight of Sample}) \times 100$$

#### Mineral Content in Fermented Mushrooms

The mineral content was determined from ash obtained at 600°C. To the 1.0 g ash sample, 5 mL of HCl and 2 mL of 5% (v/v) lanthanum chloride were added. The mixture was then stirred, boiled, and diluted to a standard volume using distilled water. Following this process, the mineral content, including zinc, iron, calcium, manganese, sodium, and potassium, was measured using an Atomic Absorption Spectrometer (Buck Scientific, Model 200, Inc., East Norwalk, Connecticut, U.S.A.) [27].

#### Fat Content in Fermented Mushrooms

Two grams of irradiated and non-irradiated mushroom samples were defatted using a chloroform: methanol (1:1, v/v) solution and processed with a Soxhlet apparatus. Afterward, 40 mg of the defatted mushrooms were weighed and placed in an ampoule containing 7.0 mL of 6 M HCl. To prevent amino acid oxidation during hydrolysis, nitrogen gas was flushed into each ampoule to remove oxygen. The ampoules were then sealed and heated at  $105 \pm 3^\circ\text{C}$  for 22 hours. After cooling, the contents were filtered at  $40^\circ\text{C}$ , producing a filtrate that was dissolved in 5 mL of acetate buffer. According to Stojković et al., (2013), the fatty acid content in mushrooms was determined using a transesterification method. Briefly, the extracted fat was dissolved in 3.4 mL of 0.5 M methanolic KOH and heated at  $95^\circ\text{C}$  for 5 minutes. Then, 3 mL of boron trifluoride (14%) in methanol was added, followed by neutralization with 0.7 M hydrochloric acid and further heating at  $90^\circ\text{C}$  for 5 minutes. Each mixture was extracted three times with n-hexane to obtain fatty acid methyl esters (FAMES). Approximately  $1 \mu\text{L}$  of the sample was injected into a gas chromatograph (GC-2010, Shimadzu, Japan), equipped with an auto-injector (AOI) and a capillary column (BPX-70). Using a standard ( $\text{C}_4\text{-C}_{22}$ ), the FAMES were quantified, and the fatty acid content was identified using GC analysis software (Shimadzu, Japan) [29]. The formula for calculating the fat percentage is shown below:

$$\text{Fat Content (\%)} = (\text{Weight of flask + fat}) - \text{Weight of flask} / \text{Sample weight} \times 100$$

#### Crude Protein Analysis

The protein content was determined using the Kjeldahl method, following the guidelines of AOAC (2010) [27]. Additionally, the AOAC method (2000) [30] was used to analyze substrate composition. For crude protein analysis, 5 g of mushroom substrate sample was weighed and placed into a 250 mL digestion tube. It was essential to ensure the digestion tube was clean to prevent any inaccuracies in protein calculation. Next, 12 mL of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) was added, and the mixture was gently swirled to ensure thorough

moistening of the sample. The digestion block was preheated to 420°C, and the digestion tube was inserted, allowing the reaction to proceed until a green-colored sample formed. Finally, the blue-green solution was titrated with 0.01 N HCl until the color changed back to its original red. The protein content was then calculated using the following formula:

$$\text{Crude Protein (\%)} = \% \text{N} \times 6.25$$

### Moisture Content in Fermented Mushrooms

Based on the guidelines provided by the AOAC method (2010), a hot air oven was used to determine moisture content. In this study, the M720 chamber (Binder GmbH, Tuttlingen, Germany) was used to dry the product until a constant weight was achieved [27].

$$\% \text{ Moisture} = 1 - \text{Dry Sample Weight} / \text{Wet Sample Weight} \times 100$$

### Carbohydrate Content in Fermented Mushrooms

Carbohydrate content was calculated using the AOAC (2010) method [27]. The carbohydrate percentage was determined using the following formula:

$$\text{CHO (\%)} = [100 - \% \text{ Moisture} - \% \text{ Protein} - \% \text{ Fat} - \% \text{ Ash}]$$

### Crude Fiber Analysis

Crude fiber content in oyster mushrooms was determined using the Fibertec™ System method (2010), following AOAC (2010) guidelines [27]. A 2 g dried sample was weighed in a pre-dried capsule, then subjected to sequential boiling in 1.25% sulfuric acid, 1.25% sodium hydroxide, and 1% hydrochloric acid using a reflux system. After boiling, the sample was thoroughly washed, dried at 130°C for 2 hours, and cooled. Post-drying, the residue was weighed, then ashed in a muffle furnace at 550°C. The crude fiber percentage was calculated based on weight differences before and after chemical treatment and ashing.

$$\text{Crude Fiber Content (\%)} = \frac{W3 - (W1 \times C) - (W5 - W4 - D)}{W2} \times 100$$

Where:

W1 = Initial weight of the capsule (g)

W2 = Weight of the sample (g)

W3 = Weight of capsule + residue (g)

W4 = Weight of crucible (g)

W5 = Weight of ash + crucible (g)

C = Blank correction for capsule solubility

D = Ash of the empty capsule

Based on the guidelines provided by Miah et al. (2017), 10 g of sample was placed in a beaker and mixed with 200 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). The

mixture was then heated for 30 minutes, with intermittent additions of water to maintain a constant volume. After heating, the mixture underwent further processing to extract fiber for weighing [31]. The fiber content was determined using the following formula:

$$\text{Crude Fiber (\%)} = \frac{[\text{Dry Weight (g)} - \text{Ash Weight (g)}]}{[\text{Moisture Weight} + \text{Fat-free Sample Weight}]} \times 100$$

### Energy Content Analysis

The energy value (in kilocalories) was calculated using the following formula:

$$\text{Energy Value} = (\text{Crude protein} \times 4) + (\text{Total carbohydrate} \times 4) + (\text{Crude fat} \times 9)$$

### Statistical Analysis

All visual data obtained from the study were statistically analyzed using Microsoft Excel Worksheet 2016 and SPSS software version 25. Replicates were not performed ( $n = 1$ ) for this analysis, as preliminary trials indicated minimal variation between repeated runs, and the measurements remained consistent. Therefore, single measurements were considered sufficiently reliable for reporting in this study. This study used a full factorial experimental design to assess the combined effects of gamma radiation dose, packaging method, and storage temperature on the quality of fermented mushroom samples. The three main factors were:

- Radiation Dose: 0 kGy (control), 1 kGy, and 5 kGy (3 levels)
- Packaging Method: Sealed (S) and Unsealed (US) (2 levels)
- Storage Temperature: Refrigerated (~8°C) and Room Temperature (28°C - 30.2°C) (2 levels)

This resulted in a total of  $3 \times 2 \times 2 = 12$  treatment combinations. Each treatment group was observed over a 12-day period with quality evaluations conducted on days 0, 3, 6, 9, and 12. Parameters including color, texture, and contamination were recorded visually and digitally. This factorial approach allowed for the assessment of both main effects and interaction effects among the variables using two-way ANOVA analysis.

## 3.0 RESULTS AND DISCUSSION

### Physical Changes on Mushroom Petals Post-Radiation

Table 1 presents the physical effects on the top and bottom surfaces of small mushroom petals following gamma radiation treatment (0 kGy) under various storage conditions: room temperature with sealed packaging (RT-S), room temperature without sealed packaging (RT-U), cold temperature with sealed packaging (CT-S), and cold temperature without sealed packaging (CT-U). In samples that were not

exposed to gamma radiation, a color change from gray to yellowish was observed under certain storage conditions, as highlighted by red circles in the mushroom images. Notably, on days 9 and 12, a yellow tint was evident in fermented mushrooms stored under CT-S, as indicated by the marked areas in Table 1. These color changes were documented using a digital microscope to capture images. One primary factor contributing to the yellowing is the enzymatic activity of polyphenol oxidase (PPO), which catalyses the oxidation of phenolic compounds to quinones, leading to discoloration. This enzymatic reaction typically results in browning or yellowing, particularly in mushroom species rich in phenolic compounds [32]. Moreover, the interaction of gamma radiation with mushroom tissue can destabilize cellular integrity, leading to the degradation of pigments such as anthocyanins. Under irradiation, the disruption of cell walls and membranes can release phenolic compounds, which then undergo oxidation, resulting in a visible yellowing effect [33].

**Table 1** Surface image of the small petal of fermented mushroom without gamma radiation treatment (0 kGy) on various storage conditions

Storage	Day	Surface		Storage	Day	Surface	
		Top	Bottom			Top	Bottom
CT-S	0			CT-U	6		
CT-U	0			RT-S	6		
RT-S	0			RT-U	6		
RT-U	0			CT-S	9		
							A yellowish effect
CT-S	3			CT-U	9		
CT-U	3			RT-S	9		
RT-S	3			RT-U	9		
RT-U	3			CT-S	12		
							A yellowish effect
CT-S	6			CT-U	12		
							A yellowish effect
				RT-S	12		
				RT-U	12		

For other storage conditions observed on days 0, 3, 6, 9, and 12, no visible color change was detected in the images. Table 2 presents the physical effects following 1 kGy radiation, where a color change was observed under certain storage conditions, as

indicated by red circles. Specifically, yellowing was noted in CT-S on days 9 and 12 and in CT-U on day 12. This suggests that cold storage can extend the shelf life of fermented mushrooms, as color changes only began to appear between days 9 and 12.

**Table 2** Surface image of the small petal of fermented mushroom after 1 kGy gamma radiation treatment on various storage conditions

Storage	Day	Surface		Storage	Day	Surface	
		Top	Bottom			Top	Bottom
CT-S	0			CT-U	6		
CT-U	0			RT-S	6		
RT-S	0			RT-U	6		
							A yellowish effect
RT-U	0			CT-S	9		
CT-S	3			CT-U	9		
CT-U	3			RT-S	9		
RT-S	3			RT-U	9		
RT-U	3			CT-S	12		
							A yellowish effect
CT-S	6			CT-U	12		
							A yellowish effect
				RT-S	12		
				RT-U	12		

Similarly, Table 3 shows that after 5 kGy radiation, a yellowish tint appeared on the cut edges of mushroom petals stored in CT-S. One plausible explanation is that cold storage reduces the overall metabolic activity of the mushrooms. Lower temperatures slow down respiration rates and other metabolic processes that contribute to water loss and tissue softening. This metabolic slowdown not only prevents cell wall degradation and maintains firmness but also inhibits the growth of spoilage microorganisms, which are highly temperature-dependent [34].

**Table 3** Surface image of the small petal of fermented mushroom after 5 KGy gamma radiation treatment on various storage conditions

Storage	Day	Surface		Storage	Day	Surface	
		Top	Bottom			Top	Bottom
CT-S	0			CT-U	6		
CT-U	0			RT-S	6		
RT-S	0			RT-U	6		
RT-U	0			CT-S	9		
CT-S	3			CT-U	9		
CT-U	3			RT-S	9		
RT-S	3			RT-U	9		
RT-U	3			CT-S	12		
							A yellowish effect
CT-S	6			CT-U	12		
				RT-S	12		
				RT-U	12		

Moreover, preserving moisture content under cold storage conditions is crucial. Mushrooms, with their thin epidermal layer, are prone to rapid dehydration, a key factor in quality loss. Maintaining a cool environment minimizes moisture loss, thereby preserving cellular turgor. This moisture retention indirectly helps limit enzymatic browning, as dehydration can concentrate oxidation substrates, accelerating color changes [35].

Additionally, the gray-to-yellow color shift, indicated by red circles, could be attributed to multiple factors, including oxygen exposure within the packaging and the effects of radiation. At 1 and 5 kGy, bacterial populations may have been weakened or reduced, facilitating lactic acid fermentation by naturally occurring bacteria in the mushrooms, both in the presence and absence of oxygen. The packaging conditions also influenced the color change: originally gray, the mushrooms developed yellow hues between days 6 and 12 of storage. When comparing packaging conditions, unsealed samples exposed to 1 and 0 kGy radiation exhibited yellowing by day 12. Similarly, sealed samples stored for 12 days under 5 and 1 kGy radiation, as well as those without radiation, also displayed yellowing.

According to Kömmling *et al.*, (2017), yellowing can occur after radiation and thermal aging when mushrooms are stored in high-molecular-weight polyethylene (HMW-PE) packaging, which contains antioxidants. This color change is attributed to

trapped free radicals within the packaging [36]. HMW-PE is commonly used as a neutron radiation shield in radioactive material storage [37,38]. Oxidation in polyethylene (PE) packaging occurs when the product is exposed to radiation and oxygen, either during or after processing [36]. This supports the likelihood that yellowing in fermented mushrooms results from oxidation following radiation exposure, particularly in sealed packaging, where oxygen becomes trapped.

This suggests that sealed packaging has a greater impact on storage conditions than unsealed packaging. According to Kamal *et al.*, (2015), the enzyme PPO can reduce color intensity and oxidize samples to yellow. Sealed packaging may retain higher levels of PPO because it traps air, whereas unsealed packaging allows airflow. Consequently, unsealed packaging can help regulate oxygen and carbon dioxide levels, reducing respiration, browning, and physical damage, ultimately extending the product's shelf life [39].

### Colour Visualisation of Fermented Mushroom Petals

The color results for the top and bottom surfaces of mushroom petals influenced the storage period after radiation at 0, 1, and 5 kGy, showing significant effects ( $p < 0.05$ ) in overall color based on Post Hoc analysis. In CT-S storage with 1 kGy radiation, mushroom color exhibited a low significant effect ( $p < 0.05$ ) on days 0, 6, 9, and 12, as indicated in Table 5. Similarly, CT-S storage without radiation also obtained a low significant effect ( $p < 0.05$ ). Table 4 demonstrates that in CT-U storage, color had a highly significant effect ( $p < 0.05$ ) on days 0 and 3 compared to the lower significance observed on days 6, 9, and 12 under the same storage conditions.

**Table 4** Grayscale colour intensity profiles of fermented small petal mushrooms without radiation (0 kGy)

*Colour	Day	CT-S	CT-U	RT-S	RT-U
Top surface	0	136.04 ± 0.15 <sup>ab</sup>	154.65 ± 0.15 <sup>a</sup>	163.50 ± 0.09 <sup>a</sup>	154.32 ± 0.77
	3	125.89 ± 0.12 <sup>b</sup>	148.22 ± 0.11 <sup>ab</sup>	164.33 ± 0.10 <sup>a</sup>	158.43 ± 0.15 <sup>c</sup>
	6	144.40 ± 0.16 <sup>a</sup>	135.55 ± 0.13 <sup>b</sup>	154.90 ± 0.12 <sup>a</sup>	162.71 ± 0.80
	9	150.33 ± 0.15 <sup>ab</sup>	133.16 ± 0.14 <sup>b</sup>	136.40 ± 0.25 <sup>a</sup>	146.79 ± 0.60
	12	151.43 ± 0.12 <sup>a</sup>	156.98 ± 0.17 <sup>a</sup>	147.42 ± 0.18 <sup>a</sup>	156.29 ± 0.55
Bottom surface	0	132.43 ± 0.88 <sup>a</sup>	149.80 ± 0.22 <sup>ab</sup>	157.08 ± 0.24 <sup>a</sup>	145.15 ± 0.46
	3	122.93 ± 0.66 <sup>a</sup>	133.83 ± 0.23 <sup>bc</sup>	150.77 ± 0.45 <sup>a</sup>	148.51 ± 0.67
	6	134.83 ± 0.74 <sup>a</sup>	102.63 ± 0.45 <sup>d</sup>	133.32 ± 0.89 <sup>ab</sup>	144.05 ± 0.87
	9	139.12 ± 0.85 <sup>a</sup>	113.90 ± 0.67 <sup>cd</sup>	109.26 ± 0.68 <sup>b</sup>	129.17 ± 0.47
	12	135.41 ± 0.79 <sup>a</sup>	168.38 ± 0.88 <sup>a</sup>	143.17 ± 0.55 <sup>a</sup>	124.35 ± 0.69

a-e: different letters indicate a significant difference ( $p < 0.05$ )

\*Each pixel is represented by a single intensity value ranging from 0 (black) to 255 (white)

Indeed, RT-S exhibited a low significant effect ( $p < 0.05$ ) on days 0, 3, and 6, with effects changing after days 9 and 12. In comparison with non-irradiated samples, both RT-S and RT-U displayed a low significant effect ( $p < 0.05$ ) over the 12-day period. However, RT-S showed a high significance ( $p < 0.05$ ) on days 0, 3, and 6 after 5 kGy radiation, while the effect was lower on days 9 and 12, as shown in Table 6. These results suggest that higher storage temperatures accelerate the rate of deterioration.

**Table 5** Grayscale colour intensity profiles of fermented small petal mushroom after 1 KGy radiation treatment

*Colour	Day	CT-S	CT-U	RT-S	RT-U
Top surface	0	156.20 ± 0.67 <sup>a</sup>	118.45 ± 0.22 <sup>ab</sup>	117.38 ± 0.44 <sup>bc</sup>	115.27 ± 0.22 <sup>c</sup>
	3	113.78 ± 0.73 <sup>b</sup>	106.16 ± 0.24 <sup>b</sup>	93.34 ± 0.55 <sup>d</sup>	132.42 ± 0.16 <sup>bc</sup>
	6	121.88 ± 0.88 <sup>b</sup>	136.27 ± 0.55 <sup>ab</sup>	106.90 ± 0.61 <sup>cd</sup>	118.26 ± 0.55 <sup>bc</sup>
	9	126.61 ± 0.82 <sup>bc</sup>	144.94 ± 0.87 <sup>a</sup>	132.83 ± 0.77 <sup>ab</sup>	148.42 ± 0.28 <sup>a</sup>
	12	151.81 ± 0.23 <sup>ab</sup>	144.67 ± 0.44 <sup>a</sup>	147.22 ± 0.81 <sup>a</sup>	159.62 ± 0.74 <sup>a</sup>
Bottom surface	0	148.28 ± 0.25 <sup>a</sup>	139.27 ± 0.55 <sup>bc</sup>	127.94 ± 0.66 <sup>b</sup>	116.57 ± 0.55 <sup>c</sup>
	3	118.64 ± 0.67 <sup>b</sup>	144.33 ± 0.12 <sup>abc</sup>	127.35 ± 0.82 <sup>b</sup>	150.37 ± 0.44 <sup>ab</sup>
	6	149.63 ± 0.73 <sup>a</sup>	135.55 ± 0.14 <sup>c</sup>	139.04 ± 0.34 <sup>b</sup>	146.21 ± 0.22 <sup>b</sup>
	9	158.27 ± 0.53 <sup>a</sup>	161.84 ± 0.77 <sup>a</sup>	159.55 ± 0.56 <sup>a</sup>	163.40 ± 0.61 <sup>a</sup>
	12	153.10 ± 0.55 <sup>a</sup>	156.47 ± 0.45 <sup>ab</sup>	156.81 ± 0.67 <sup>a</sup>	158.65 ± 0.69 <sup>a</sup>

a-e: different letters indicate a significant difference ( $p < 0.05$ )

\*Each pixel is represented by a single intensity value ranging from 0 (black) to 255 (white)

**Table 6** Grayscale colour intensity profiles of fermented small petal mushroom after 5 KGy radiation treatment

*Colour	Day	CT-S	CT-U	RT-S	RT-U
Bottom surface	0	147.42 ± 0.27 <sup>abc</sup>	141.58 ± 0.22 <sup>b</sup>	160.05 ± 0.44 <sup>a</sup>	155.15 ± 0.22 <sup>c</sup>
	3	125.05 ± 0.66 <sup>c</sup>	129.63 ± 0.68 <sup>b</sup>	116.51 ± 0.57 <sup>c</sup>	125.05 ± 0.81 <sup>c</sup>
	6	148.87 ± 0.22 <sup>ab</sup>	157.62 ± 0.33 <sup>a</sup>	138.88 ± 0.88 <sup>b</sup>	136.12 ± 0.76 <sup>c</sup>
	9	127.42 ± 0.87 <sup>bc</sup>	159.35 ± 0.54 <sup>a</sup>	149.37 ± 0.67 <sup>ab</sup>	123.50 ± 0.22 <sup>c</sup>
	12	166.85 ± 0.77 <sup>a</sup>	155.50 ± 0.78 <sup>a</sup>	148.14 ± 0.23 <sup>ab</sup>	153.82 ± 0.81 <sup>c</sup>

a-e: different letters indicate a significant difference ( $p < 0.05$ )

\*Each pixel is represented by a single intensity value ranging from 0 (black) to 255 (white)

Additionally, room temperature throughout the experiment likely contributed to mushroom deterioration when stored for extended periods [40]. To extend shelf life, a radiation dose of 4.5 kGy/h is recommended over a higher dose of 32 kGy/h. Furthermore, a 5 kGy radiation treatment reduced PPO enzyme activity by 93%, supporting its suitability for mushroom storage beyond the 12-day study period [41]. This substantial reduction in PPO activity is likely due to the ionizing effects of gamma radiation on the enzyme's molecular structure. Ionizing radiation generates free radicals that can attack peptide bonds, induce oxidative modifications, and disrupt the integrity of key secondary structures, such as the four-helix bundle that forms the core of PPO. These structural perturbations can lead to enzyme misfolding or denaturation, impairing its catalytic properties and reducing browning reactions. Previous studies have similarly demonstrated that nonthermal irradiation treatments at a 5 kGy dose significantly reduce PPO activity [42,43,44].

Moreover, this experiment was conducted at room temperatures ranging from approximately 28 to 30.2°C. According to Jabłońska-Ryś & Stawinska (2012), mushrooms can last up to 18 days at 20°C [41], whereas Liu *et al.*, (2015) found that mushrooms remain viable for only 7 days at temperatures between 21 and 22°C [43]. As temperature increased, the metabolic rates accelerate, leading to increased respiration, microbial proliferation, and oxidative stress, which may counteract the protective effects of reduced browning enzyme

activity. Study have shown that while controlled treatments, such as irradiation combined with modified atmosphere packaging, can effectively extend shelf life, storage at room temperature remains problematic due to these compounded factors. Over time, this leads to textural degradation and compositional changes [44]. In conclusion, the experimental room temperature conditions used in this study were unsuitable for long-term storage. Instead, cold temperatures are recommended to extend the shelf life of fermented mushrooms.

### Texture Visualization

Texture data were also collected visually, with an average rating on a scale of 1 (non-watery) to 2 (watery), including intermediate values such as 1.33 and 1.67 to indicate slight wateriness. The data revealed a significant effect ( $p < 0.05$ ) on all radiation levels (0, 1, and 5 kGy). Post Hoc analysis further indicated significant differences across all storage conditions, namely RT-S, RT-U, CT-S, and CT-U in the non-irradiated samples; CT-S, CT-U, and RT-S at 1 kGy; and CT-S, RT-S, and RT-U at 5 kGy, as shown in Tables 7, 8, and 9. However, RT-U at 1 kGy and CT-U at 5 kGy did not show a significant effect ( $p > 0.05$ ) on mushroom texture.

**Table 7** Texture effects on the fermented mushroom without radiation at a dose of 0 kGy

Day	CT-S	CT-U	RT-S	RT-U
0	1	1	1	1
	1	1	1	1
	1	1	1	1
3	1	1	1	1
	1	1	1	1
	1	1	1	1
6	1	2	2	2
	2	2	2	1
	1	2	1	2
9	1	2	2	2
	1	2	1	2
	1	2	1	1
12	1	2	2	2
	1	2	2	2
	1	2	2	2

Scale 1: Non-watery; Scale 2: Watery

**Table 8** Texture effects on the fermented mushroom at a dose of 1 kGy

Day	CT-S	CT-U	RT-S	RT-U
0	1	1	1	1
0	1	1	1	1
0	1	1	1	1
3	1	1	1	2
3	1	1	1	2
3	1	1	1	2
6	2	2	1	2
6	1	2	1	2
6	2	1	1	2
9	2	2	1	2
9	2	2	1	2
9	1	2	2	2
12	2	2	1	2
12	2	2	1	2
12	2	2	1	2

Scale 1: Non-watery; Scale 2: Watery

**Table 9** Texture effects on the fermented mushroom at a dose of 5 kGy

Day	CT-S	CT-U	RT-S	RT-U
0	1	1	1	1
0	1	1	1	1
0	1	1	1	1
3	1	1	2	2
3	1	1	2	2
3	1	1	2	1
6	1	1	1	2
6	1	1	1	2
6	1	1	1	1
9	1	1	1	1
9	1	2	2	2
9	1	1	2	2
12	2	2	2	2
12	2	2	2	2
12	2	2	2	2

Scale 1: Non-watery; Scale 2: Watery

Therefore, in this phase, selecting an unsealed storage option appears beneficial for maintaining texture. In sealed packaging, ambient oxygen can become trapped, accelerating oxidative reactions that may compromise the mushroom's texture and color. Kamal *et al.* (2015) demonstrated that the respiratory gases in oyster mushrooms packaged with different sealable polymeric materials significantly impacted their shelf life. Their study indicated that sealed packaging could trap oxygen, leading to oxidation that affects intrinsic quality attributes. In contrast, unsealed packaging allows continuous exchange of respiratory gases, reducing the concentration of oxygen in the headspace and minimizing oxidative damage [39].

Fresh mushrooms can last up to four weeks when stored at a controlled, refrigerated temperature, making cold storage a suitable method for maintaining quality over an extended period. The primary reason for this is that under cold storage conditions, specifically around 4°C, metabolic activities, enzymatic reactions, and microbial growth rates are substantially slowed [45].

### Contamination Visualization

No contamination was observed in the fermented mushrooms following radiation at 1 and 5 kGy or in the non-radiated samples (0 kGy), as shown in Table 10. This was confirmed visually by the absence of black or white spots. Typically, microbial contamination in vegetables causes visible changes, such as black, gray, or pink discoloration on leaf surfaces [40]. In this study, cold storage was found to be effective in extending the shelf life of fermented mushrooms. According to Rawat (2015), refrigeration delays the microbial lag phase and slows microbial growth [40]. Additionally, no visible mold growth was observed during the storage period, indicating the effectiveness of refrigeration in preserving the product's quality.

In fermented mushrooms, an additional inhibitory effect is exerted by the decreased pH that develops during fermentation. During acid-producing fermentations, bacteria such as *Lactobacillus*

convert carbohydrates into organic acids, thereby lowering the pH of the substrate [46]. This acidic environment selectively inhibits the proliferation of microbes that lack acid tolerance while promoting the growth of acidophilic species. The synergistic effect of refrigeration and acidification plays a crucial role in food preservation. While refrigeration slows down microbial physiological processes by prolonging the lag phase [47], acidification, whether naturally occurring or process-induced which further enhances preservation by directly inhibiting microbial enzymes and disrupting cell membrane functions [46,48].

**Table 10** Visual contamination at doses of 0, 1, and 5 kGy

Day	CT-S	CT-U	RT-S	RT-U
0	0	0	0	0
3	0	0	0	0
6	0	0	0	0
9	0	0	0	0
12	0	0	0	0

Scale 0: No foreign contamination and fungal growth were visualized

### Proximate Analysis of Fermented Mushrooms between 0 kGy (Control) and 5 kGy Radiation

Table 11 presents the overall results for the control and 5 kGy radiated mushrooms, obtained using various analytical methods as follows.

**Table 11** Proximate analysis of fermented mushroom sample at 0 kGy

Analysis	Unit	Results	Methodology/ Equipment/ Technique
Ash	g/100g	2.1	STP/Chemistry/A05 based on AOAC 20 <sup>th</sup> Edition: 923.03
Moisture	g/100g	82.8	STP/Chemistry/A04 based on AOAC 20 <sup>th</sup> Edition: 950.46
Fat	g/100g	0.4	STP/Chemistry/A02 based on AOAC 20 <sup>th</sup> Edition: 991.36
Protein	g/100g	3.3	STP/Chemistry/A03 based on AOAC 20 <sup>th</sup> Edition: 981.10
Carbohydrate	g/100g	11.4	STP/Chemistry/A06 based on Promerance Food Analysis: Theory and Practical, 2 <sup>nd</sup> Edition (p. 637)
Energy	kcal/100g	62	STP/Chemistry/A01 based on Pearson's The Chemical Analysis of Foods (6 <sup>th</sup> Edition, p. 578)
Crude fiber	g/100g		STP/Chemistry/A08 based on AOAC 20 <sup>th</sup> Edition: 962.09
Ash	g/100g	2.5	STP/Chemistry/A05 based on AOAC 20 <sup>th</sup> Edition: 923.03
Moisture	g/100g	80.3	STP/Chemistry/A04 based on AOAC 20 <sup>th</sup> Edition: 950.46
Fat	g/100g	0.4	STP/Chemistry/A02 based on AOAC 20 <sup>th</sup> Edition: 991.36
Protein	g/100g	4.1	STP/Chemistry/A03 based on AOAC 20 <sup>th</sup> Edition: 981.10
Carbohydrate	g/100g	12.7	STP/Chemistry/A06 based on Promerance Food Analysis: Theory and Practical, 2 <sup>nd</sup> Edition (p. 637)
Energy	kcal/100g	71	STP/Chemistry/A01 based on Pearson's The Chemical Analysis of Foods (6 <sup>th</sup> Edition, p. 578)
Crude fiber	g/100g	<0.1	STP/Chemistry/A08 based on AOAC 20 <sup>th</sup> Edition: 962.09

### Ash Content in Fermented Mushroom

The ash content in mushrooms irradiated at 5 kGy was recorded at 2.5%, compared to 2.1% in the control sample. Ash represents the primary residue of

mineral composition, indicating the total mineral content in a food product [49]. These results suggest that irradiated mushrooms yield a higher ash value than non-irradiated mushrooms, indicating improved mineral retention in the 5 kGy-treated sample. According to Kortei *et al.*, (2017), statistical analysis showed that the ash content in irradiated dried mushrooms ranges from 6.16% to 8.31%, with no significant difference ( $p > 0.05$ ) [50].

This increase in ash content may be attributed to the physicochemical modifications induced by gamma irradiation. One possible explanation is that irradiation can cause partial dehydration and the breakdown of certain organic compounds, leading to a relative enrichment of inorganic residues, which are quantified as ash during proximate analysis [51].

### Fat Content in Fermented Mushroom

The study results indicate that the fat content in both irradiated and non-irradiated fermented mushrooms remained approximately 0.4%, suggesting that radiation had no significant ( $p > 0.05$ ) effect on fat content. This finding aligns with the naturally low-fat composition of mushrooms, which are cholesterol-free and contain minimal fatty acids [50]. This characteristic is beneficial for human health and dietary intake.

Kortei *et al.* (2017) observed that the fat content in irradiated mushrooms typically range from 0.6% to 3.2%, indicating that radiation can have varying effects depending on the mushroom type and processing conditions [52]. However, in this study, the fat content remained unchanged, further confirming that gamma irradiation does not degrade fats in mushrooms. The consistency in fat levels across both irradiated and non-irradiated samples not only demonstrates that the irradiation process is non-destructive to lipids but also reinforces the fact that mushrooms naturally contain low levels of fat.

### Crude Fiber Content in Fermented Mushroom

The crude fiber analysis for both 5 kGy-irradiated and 0 kGy (control) mushrooms showed a content of < 0.1%, suggesting no significant change in fiber content between the irradiated and control samples. Studies by Musieba *et al.* (2013) and Egwim *et al.* (2011) reported crude fiber values ranging from 3% to 32%, indicating that the fiber content observed in this study falls below these previously reported ranges, suggesting a lower fiber content in the tested mushroom samples [53,54]. Meanwhile, Cardoso *et al.* (2019) demonstrated that while gamma irradiation affects various biochemical properties, the overall macromolecular structures associated with dietary fiber, particularly cell wall polysaccharides, remain relatively stable. This stability is likely due to the inherent resistance of cellulose and chitin complexes in the mushroom cell wall to free radical-induced degradation at moderate irradiation levels [55].

### Crude Protein Content in Fermented Mushroom

In this study, the protein content in 5 kGy-irradiated mushrooms was 4.1%, compared to 3.1% in the control sample, indicating a slight increase in protein content with radiation. According to Kortei *et al.*, (2017), at 0 months of storage, protein content in mushrooms typically ranges from 12.51% to 15.25% [50]. This increase in protein content can be explained by several interrelated factors. Gamma irradiation is known to reduce microbial spoilage and may induce cell wall disruption, thereby enhancing the extractability of intracellular proteins. This mechanism could lead to a relative increase in measured protein concentration, as the breakdown of cellular structures makes proteins more accessible for analysis [55]. Additionally, Andrade *et al.* (2014) reported that a moderate dose of irradiation increased the crude protein content in *Agaricus bisporus* strains cultivated in various composts, suggesting that irradiation may alter the metabolic or structural properties of proteins, making them more detectable during analytical procedures [56].

### Moisture Content in Fermented Mushroom

The moisture content in 5 kGy-irradiated mushrooms was 80.3%, slightly lower than the control sample's 82.8%. This difference may be attributed to the effects of ionizing gamma radiation on the cellular structure of mushrooms. Gamma irradiation at moderate doses, such as 5 kGy, is known to generate free radicals that can disrupt cell membranes and alter water-binding sites within the tissue [55]. These disruptions may facilitate the migration or evaporation of free water, effectively reducing the overall moisture content, even if the change is relatively minor.

Furthermore, Kortei *et al.* (2017) reported that from 0 to 3 months of storage, moisture content in dried mushrooms showed no significant difference ( $p > 0.05$ ), increasing from 14.11% to 15.80%, and further to 16.11% between 6 and 12 months [52]. The variation in moisture content among mushrooms is largely influenced by different processing methods. While fermented mushrooms retain higher moisture levels than dried mushrooms, irradiated mushrooms in this study exhibited lower moisture content compared to non-irradiated samples.

Moisture content in mushrooms also varies depending on factors such as harvesting, cultivation, culinary preparation, and storage conditions [57]. Additionally, the mushroom's outer structure, protected by a porous epidermis, plays a crucial role in hydration [58]. Singer (1986) noted that mushroom quality declines due to the easily dehydrated epidermal layer, making moisture retention a key factor in maintaining freshness [59].

### Carbohydrate Content in Fermented Mushroom

The carbohydrate content in 100 g of mushrooms irradiated at 0 kGy was 11.4%, whereas mushrooms irradiated at 5 kGy contained 12.7%, indicating a slight increase in carbohydrate content with radiation. This suggests that 5 kGy irradiation enhances the detectable carbohydrate content compared to the control. According to Kortei et al., (2017), the carbohydrate content in irradiated dried mushrooms ranged from 61.39% to 65.50% after three months of storage at 1 to 2 kGy [50].

Ionizing radiation is known to interact with biological matrices by inducing bond cleavage and molecular rearrangements, potentially leading to the liberation of simple sugars from more complex polysaccharides [60]. One possible explanation for the observed increase in carbohydrate content is the disruption of cell wall components in irradiated mushrooms. This disruption may facilitate the conversion of structural polysaccharides into soluble forms, making them more readily detectable in chemical assays [56].

Furthermore, the process of radiolysis induced by gamma irradiation generates free radicals that may cleave glycosidic bonds in complex carbohydrates [60,61]. Such cleavage reactions can convert large polysaccharides into smaller oligosaccharides or monosaccharides, which are more easily extractable and quantifiable [62,63]. Studies on similar food matrices have demonstrated that irradiation can modify both the structure and solubility of carbohydrate polymers, thereby affecting the net measured carbohydrate content [56].

### Energy Content in Fermented Mushroom

Irradiated dried mushrooms contain energy levels ranging from 247.8 to 284.9 kcal/100 g after storage under various conditions. The data indicates no significant effect ( $p > 0.05$ ) of storage on metabolic energy. The relatively narrow range of energy values suggests that the structural compounds responsible for energy provision such as carbohydrates, proteins, and fats remain largely intact and are not significantly degraded or modified during storage [64].

After three months of storage in polyethylene and polypropylene packaging, no significant effect ( $p > 0.05$ ) was observed, with energy levels ranging from 273.7 to 282.1 kcal/100 g in polyethylene and 274.4 to 284.6 kcal/100 g in polypropylene. The energy content in mushrooms irradiated at 0 kGy and 5 kGy was 62 and 71 kcal/100 g, respectively, after 12 days of storage. This suggests that irradiation induces biochemical changes that may lead to the breakdown or modification of macromolecules, potentially generating simpler compounds that are more readily metabolized or quantifiable as energy [65,66].

## 4.0 CONCLUSION

This study confirms that gamma radiation, particularly at a 5 kGy dose, significantly improves the shelf life and nutritional quality of fermented mushrooms known as Pekasam Cendawan. When paired with cold temperature unsealed storage, this treatment effectively reduces microbial growth, maintains texture, and prevents undesirable color changes. The 5 kGy dose preserved essential nutrients, including protein at 4.1 percent and carbohydrates at 12.7 percent, with minimal moisture loss at 80.3 percent. These results highlight gamma irradiation as a safe and efficient method for preserving fermented mushrooms, meeting consumer demand for high-quality, minimally processed, and microbiologically safe foods, and offering strong potential for commercial adoption.

## 5.0 RECOMMENDATION

Future studies should explore the long-term impact of irradiation on the sensory attributes of fermented mushrooms and assess consumer acceptability. Additionally, optimizing radiation parameters to further minimize texture modifications while maximizing preservation benefits will be crucial for broader industrial adoption. Overall, this study presents a sustainable and scientifically validated approach to enhancing the economic value, safety, and nutritional integrity of fermented mushrooms through advanced preservation technologies.

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## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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