IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 4 issue 2 May, 2025 Research Article

Effect of Mutagens (Sodium Azide and Cobalt-60) on the Nutritional Properties of Cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871

Alfa, Bumojo Micah[1]*, Musa, Hannatu[2] and Adelanwa, M. A[3].
[1*2]Department of Botany, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[3]Department of Botany, Federal University, Lokoja, Kogi State, Nigeria.
***Corresponding Author:** *bumojomicahalfa@gmail.com*

ABSTRACT

Pleurotus ostreatus (Jacq.) P. Kumm. 1871 is a widely cultivated edible mushroom known for its high nutritional and therapeutic value. However, its nutrient composition is often inconsistent due to environmental and substrate variability. This study investigated the potential of induced mutagenesis using sodium azide (NaN_3) and cobalt-60 (^{60}Co) gamma irradiation to enhance the nutritional properties of *P. ostreatus*. Spawn samples were treated with different concentrations of NaN_3 at 50, 100, 200, and 300 ppm for 30 minutes and irradiated with ^{60}Co at 9.4722 µGy/s for 3, 6, 12, and 24 minutes. Treated spawns were cultivated on sterilized corn cob–rice bran substrates (70:30), incubated under harmattan conditions with temperature and relative humidity of 18–38°C; and 20–58% RH respectively, and harvested after fruiting (approximately 28 days).. Proximate analyses were conducted using international standard methods to assess dry matter, crude fiber, protein, oil, ash, and carbohydrates. Significant increases were observed in crude fiber from 9.26 to 13.44 g/100 g (12 min irradiation), oil from 1.13 to 1.92 g/100 g (300 ppm NaN_3), and ash from 10.41 to 16.13 g/100 g (12 min irradiation), with p-values < 0.05, while dry matter, protein, and carbohydrate contents remained statistically unchanged. This research addresses the problem of inconsistent nutrient profiles in *Pleurotus ostreatus* and justifies the application of mutagenesis to achieve targeted nutritional enhancement. The findings suggest that low-dose NaN_3 and ^{60}Co treatments can serve as effective tools for nutrient enrichment of this edible mushroom. It is recommended that further molecular and toxicological evaluations be conducted to validate the genetic stability and safety of treated strains before commercial deployment.

Keywords: Enhancement, *Pleurotus ostreatus*, Oyster Mushroom, Sodium Azide, Cobalt-60 Irradiation, Proximate Composition,.

Introduction

A mushroom is the conspicuous sexual structure of a fungus (Pant *et al*., 2020) that is made up of a stipe, pileus, lamellae and produces spores from the underside of the pileus (Masarirambi *et al*., 2011). About 2-11 million species of fungi have been estimated (Phukhamsakda *et al*., 2022) out of which only 155, 885 species have been formally described (Species fungorum, 2023) which includes chytridiacious fungi, lichen-forming fungi, slime molds, chromistan fungi, yeast, molds and mushroom-producing filamentous fungi. About 14, 000 to 22, 000 are mushroom-producing species. As at 2023, the number of species of *Pleurotus* in the Species Fungorum stands at 583 (Species Fungorum, 2023).

These edible basidiomycetes are well known worldwide as oyster mushroom, and in terms of production of edible mushrooms, it occupies the third position, behind the genus *Agaricus* and *Lentinula* (Zeng *et al*., 2022).

Pleuratus ostreatus (Jacq.) P. Kumm. 1871, commonly referred to as the oyster mushroom, is one of the most widely cultivated edible fungi in the world due to its rapid growth, high nutritional value, and potential health benefits. Known for its delicate texture, mild flavor, and versatility in cooking, *P. ostreatus* is rich in essential nutrients, including proteins, vitamins, minerals, and dietary fiber, making it a valuable dietary supplement (Kumar *et al.,* 2018).

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Additionally, *P. ostreatus* contains bioactive compounds with antioxidant, anti-inflammatory, and anti-cancer properties, which have attracted significant attention in food science and medicinal research (Kim *et al*., 2021).

Sodium azide (NaN_3), a chemical compound often used as a mutagen in plant and fungal research, has demonstrated potential in enhancing the growth and biochemical properties of various crops and fungi (Ali *et al*., 2019). It is known to induce genetic mutations that can lead to desirable traits such as increased resistance to diseases or enhanced nutritional content. Cobalt-60 (Co-60), a radioactive isotope, is commonly utilized for food irradiation, a technique that has been widely adopted for its ability to sterilize, reduce microbial load, and extend the shelf life of perishable products (Sánchez *et al.*, 2020). Irradiation can also influence the biochemical composition of edible fungi, altering their antioxidant properties, texture, and flavor, which may improve both their nutritional value and shelf stability.

The nutritional content of *Pleurotus ostreatus* is highly variable, readily influenced by a wide array of factors. These factors include, but are not limited to, the quality, type, and quantity of the substrate, and the prevailing environmental conditions (Melanouri *et al*., 2024; Preprints.org, 2025; Soliman *et al*., 2022). Fortunately, mutation offers a pathway to bypass these limitations and develop strains with superior nutritional content (Al-Salihi & Alberti, 2021; Choudhary *et al*., 2021). The effects of NaN_3 and ^{60}Co have not been extensively studied, especially in terms of how these treatments impact's the nutritional properties of *P. ostreatus*, a mushroom species that differs in many aspects from other commonly studied mushrooms such as *Agaricus bisporus* (button mushrooms) and *Lentinula edodes* (shiitake). This lack of knowledge presents a research opportunity to explore how NaN_3 and ^{60}Co could potentially improve the nutritional quality of *P. ostreatus*.

Recent literature highlights the positive impact of irradiation on the antioxidant capacity and shelf life of mushrooms, with some studies indicating that moderate levels of gamma irradiation can enhance the stability of vitamins, increase bioactive compounds, and delay spoilage (Mala *et al*., 2020; Martínez *et al*., 2021).

Furthermore, mutagenesis using sodium azide has been shown to produce genetically modified strains with altered nutrient profiles, potentially increasing the mushroom's nutritional content (López *et al*., 2022). However, the effects of ^{60}Co on cultivated *P. ostreatus* remain underexplored. This research seeks to evaluate the effects of NaN_3 and ^{60}Co irradiation on the nutritional properties of *Pleurotus ostreatus*. In this study, sodium azide (a chemical mutagen) and ^{60}Co (a physical mutagen) were applied individually to mushroom cultures. Although the treatments were not combined, a two-way ANOVA was employed to assess the main effects and interaction effects between mutagen type and treatment levels on nutritional parameters. This approach enabled the evaluation of potential synergistic or differential impacts without the need for physically combined treatments.

Materials and Methods

Study Area

Pleurotus ostreatus spawns was collected from Mushroom Research and Training Laboratory, Yaba College of Technology Lagos State, Nigeria. Corn (*Zea mays* L.) cob and Rice bran was collected from Farmers in Zaria, Kaduna State, Nigeria. The research was conducted at the Mycology Laboratory of the Department of Botany, Ahmadu Bello University, Zaria.

Mushroom Spawn Irradiation

Gamma irradiation dosage selection Criteria/ Protocol for *P. ostreatus*

Based on reports that fungi tend to become radio-resistant upon exposure to high/chronic levels of irradiation by Dadachova *et al*., 2008 and Dighton *et al*., 2008, the preference for a low/acute dose was initiated. Following reports by kovalchuk *et al*. (2000) of the mutagenic potency of low dose of irradiation though at chronic levels (about 10 months) of 0.1-0.3 Gy in inducing germ line mutation in wheat and also the potency of ultra-low dose of 0.00000534 Gy/day (5.34 µGy/day) in stimulating defense/stress response in rice leaves (Rakwal *et al*., 2009), a dosage within this range was adopted.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Spawns of *Pleurotus ostreatus* for this present study were placed in well-labeled, transparent, round plastic containers with lids, each with a capacity of 100 mL. The containers were securely sealed and transported to the Centre for Energy Research and Training (CERT), Ahmadu Bello University, Zaria, Nigeria, for irradiation.

Irradiation was carried out using a ^{60}Co gamma radiation source with an activity of 1110×10^6 Bq. The online Calculator, Radprocalculator.com (2021) was used for the dosage calculation. 9.4722 µGy/seconds (equivalent to 0.0341 Gy/hour) was achieved by placing the mushroom spawns 10 cm away from the ^{60}Co gamma source. This dose rate remained constant but at different duration for different treatment (T). The same dosage was observed for T1 was for 3 minutes of exposure, T2 for 6 minutes, T3 for 12 minutes and T4 was for 24 minutes of exposure was utilized for *P. ostreatus*. For screening for lethal dose, the spawns were exposed to varying dosage with maximum of 100 Gy of 60Co for *P. ostreatus.* When cultured for mycelial growth, they all had their mycelial growth on the 3rd day thereby indicating their resistance to high levels of radiation.

All treatments were conducted under consistent environmental and handling conditions to ensure the reliability and reproducibility of the experiment. After irradiation, the spawns were stored in the refrigerator (temp < 15^{O} C) for subsequent culturing and analysis.

Mushroom Spawn Treatment with Chemical Mutagen

Treatment with Sodium Azide was done according to the method described by Gehan (2011) as described below:
Spawns of *P. ostreatus* was soaked in Sodium Azide (NaN_3) solutions with the concentrations of 0, 100, 200, 300 and 50 PPM (Part per Million) for 30 Minutes after carrying out a preliminary study for the lethal dose and an effective duration. 100, 200, 300, and 50 PPM was prepared by dissolving 0.1g, 0.2g, 0.3g and 0.05g of Sodium Azide weighed by using the American Digital pocket weighing scale 600G × 0.1G (MS-600-BLK) respectively in 1 Liter of distilled water. The pH of the solution was adjusted to 3.6 using orthophosphoric acid. Each solution was labeled accordingly.

Three weeks old mycelium (spawns) of the fungi; *P. ostreatus* was subjected to each of these concentrations with exception of 0.5 g/L for 30 minutes, 1 hour, 2 hours, 3 hours and 4 hours. There was no mycelial growth in *P. ostreatus* till up to 90 days in all the experimented durations for 0.4 g/L (400 PPM) of NaN_3, thus 0.4 g/L was considered as the lethal dose and was replaced with 0.05 g/L (50 PPM) for *P. ostreatus.* Therefore 30 minutes was chosen as the minimum effective duration because it had a lethal impact on *P. ostreatus* at 0.4 g/L of NaN_3.

Experimental Design

This research seeks to evaluate the individual effect of sodium azide and cobalt-60 irradiation on the nutritional properties of *Pleurotus ostreatus*. The experimental design was a completely randomized design, designed to study the effect of two (2) independent variables (physical and chemical mutagens) and one (1) negative control variable (neither exposed to physical nor chemical mutagens) on the nutritional properties of *P. ostreatus*. The physical mutagen of ^{60}Co gamma source at 9.4722 µGy/seconds (equivalent to 0.0341 Gy/hour) has four (4) levels of treatment; 3minutes (T1), 6 minutes T2), 12 minutes (T3) and 24 minutes (T4) of exposure to gamma (G) rays while the chemical mutagen also has four (4) levels of treatments 50 PPM (T5), 100 PPM (T6), 200 PPM (T7) and 300 PPM (T8) of Sodium Azide, with 3 replicates for each levels (treatments). This design consists of, 8 experimental groups (4 treatment levels for physical and another 4 treatment levels for chemical mutagen), 1 negative control group with three (3) replicates for *P. ostreatus*, thereby making a total of 27 experimental units.

Substrate Preparation and Mushroom Cultivation

The corn cob was air dried for one week and then ground into tiny bits (1.2cm). Rice bran was also air dried for one week. Sterile transparent bottles of dimension (7.5×17×7.5 cm) were used as substrates containers. Chopped Corn cob (70%) supplemented with Rice Bran (30%) was used as substrates. 140g (70%) of the chopped-Corn cob and 60g (30%) of rice bran was measured into one sterile bottle. This Process was repeated for fifty-three (53) other sterile bottles. 560g of Calcium Carbonate ($CaCO_3$) was dissolved in 14 liters (1400ml) of distilled water and stirred.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Twenty five milliliter (25ml) of the $CaCO_3$ solution was added to each of the 54 substrates bottles, the substrates were left to imbibe the $CaCO_3$ solution for 30 minutes with the substrates bottle covered. After 30 minutes the substrates bottles were turned upside down so as to drain away excess water.

Steam sterilization of the substrates and Inoculation

This was done by modifying the method described by Chukwurah *et al*. (2012). The substrates were placed on a wooden stand of 60 cm long inside a metal drum filled up to one-fifth (1/5) its volume with water such that the substrates bottles on the stands are 25 cm above the level of the water for steaming purpose. The substrates bottle was steam sterilized at the temperature of 100°C for 7 hours at a nearby location outside the Mycology Laboratory in the Department of Botany, Ahmadu Bello University Zaria and allowed to cool for 24 hours before they were moved into the Laboratory for inoculation.

Holes were aseptically created within the substrate in the bottles and inoculation was done by using an inoculation hook to place spawns into the holes created in the substrate. Untreated spawns were used for inoculation in the negative control. The cultures were then incubated at room temperature for 2 weeks. The bottles were opened for fruiting and sprinkled with water daily. After 6 days of watering, primordial initiation was observed.

Growth Conditions and Harvesting

This Study was conducted during the harmattan when the relative humidity was low (20-58 %) and the temperature was between 18-30° C at night and 18-38° C during the day. Harvesting was carried out 3 days after initiation. The fruiting bodies were weighed and were air-dried for 2 weeks in the Laboratory. The low temperature and humidity of the harmattan quickened the drying of the mushrooms.

Proximate Analysis

The proximate analysis was carried out at the Biochemical Laboratory of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria Nigeria.

The standard methods of Association of Analytical Chemist (AOAC, 2000) were adopted for the determination of Dry Matter, ash content, Crude Fibre, crude protein, Oil and Carbohydrate. Carbohydrate content was estimated based on the net difference between the other nutrients and total percentage composition.

Determination of dry matter

The percentage moisture and dry matter content was determined with the aid of the laboratory oven, from the differences between the fresh and dry weight of the samples using the following equation (Rahman *et al*., 2012).

$$\text{Moisture (\%)} = \frac{W1-W2}{\text{Weight of sample}} \times 100$$

Dry Matter (%) = 100 – Moisture (%)

W_1 = Fresh weight

W_2 = Oven Dried Weight

Crude fiber determination

After determining the ash content in mushrooms (2 g of it was placed in a crucible and set in a muffle furnace at 500 °C for 12 h, after which it was removed, cooled in a desiccator and weighed). Thereafter, 0.5 g of dried sample was extracted using acetone in a 50 ml centrifuge tube, the extract containing fat was discarded, and 50 ml of H_2SO_4 (1.25%) was added, the mixture was boiled at 100 °C for 15 min, centrifuged at 1000 rpm, then decanted and the solution was discarded (AOAC, 2000, method 962.09). This was followed by the addition of 50 ml NaOH (1.25%), boiling at 100 °C for 15 min, centrifugation at 1000 rpm, decantation, discarding the solution, addition of 25 ml H_2SO_4 (1.25%), decanting of solution, addition of 50 ml H_2O, filtration of the solution on a pre-weighed filter paper using Buchner, washing with two H_2O portions of 50 ml each and then using 25 ml ethanol portion. The residue was dried for 2 h at 130 °C in oven; the remaining residue represented fiber and ash contents.

The percentage of fiber was then calculated using the formula below:

$$\% \text{ Fiber} = \frac{Weight\ (paper + residue) - Weight\ (paper)}{\text{Weight (sample)} - \%\ crude\ ash}$$

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Determination of protein content

A quantity (0.5-1g) of the ground mushroom sample was placed in a digestion flask. 5g of Kjedhal catalyst and 200 ml of conc. H_2SO_4 was added to the sample (AOAC, 2000). A blank was prepared by placing the above chemical in a tube with exception of the sample. The flask was placed in an inclined position and was heated gently until frothing ceased. The solution was then boiled briskly until solution clears. The solution was cooled and 60 ml of distilled water was added cautiously. Thereafter the flask was immediately connected to the digestion bulb on the condenser, with the tip of the condenser immersed in standard acid and 5-7 drops of mixed indicator in the receiver. The flask was rotated to mix contents thoroughly and then heated until all NH_3 was distilled. The receiver was removed, the tip of the condenser was washed and the excess standard acid was titrated against standard NaOH solution.

Calculation:

$$\text{Protein (\%)} = \frac{(A-B)\times N \times 14.007 \times 6.25}{W}$$

Where A = Volume (ml) of 0.2 N HCl used in the sample titration
B = Volume (ml) of 0.2 N HCl used in blank solution
N = Normality of HCl
W = Weight (g) of sample
14.007 = Atomic weight of Nitrogen
6.25 = the protein-nitrogen conversion factor for fish and it's by product

Determination of ash content

The crucible and the lid were placed in the furnace at 550^{O}C overnight to ensure that impurities on the surface of the crucible are burned off (AOAC, 2000). The crucible was cooled in the desiccator (30 min). The crucible and the lid were weighed to 3 decimal places. About 5g of the ground mushroom sample was weighed into the crucible and heated over low Bunsen flame with lid half covered. After the fumes ceased crucible and lid was placed in the furnace. The sample was heated at 550 ^{O}C overnight. Care was taken not to cover the lid during heating until after heating so as to prevent the loss of fluffy ash. Thereafter it was cooled down in the desiccator. The ash together with the crucible and lid were weighed when the samples turned to gray.

Calculation

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Determination of oil content

The bottle and the lid were placed in the incubator at 105^{O}C overnight so as to ensure that the weight of the bottle is stable. About 3-5 g of the mushroom sample was weighed into the paper filter and wrapped (AOAC, 2000). The mushroom sample was transferred into the extraction thimble and then into the soxhlet. The bottle was filled with about 250 ml of Petroleum ether and then taken to the heating mantle. The sample was heated for about 14 hours. The solvent was evaporated by using the vacuum condenser. The bottle was incubated at 80-90^{o}C until the solvent completely evaporated and the bottle became dried. The bottle was transferred into the desiccator to cool with a partially covered lid. The bottle and its dried content were then reweighed.

Calculation

$$\text{Fat (\%)} = \frac{\text{Weight of Fat}}{\text{Weight of sample}} \times 100$$

Carbohydrate content

This was estimated by calculating the Nitrogen Free Extract (NFE) and the Total Carbohydrate content. These two Parameters give information about the carbohydrate content of the Mushrooms (Aishah and Wan Rosli, 2013)

Nitrogen free extract (NFE)

This is an estimate of the Carbohydrate content of the mushroom. It was estimated based on the net difference between the other nutrients and total percentage composition.

Nitrogen Free Extract (NFE %) = 100 − (CP + CF + OIL + Ash).

Total carbohydrate (TC)

This is cumulative of the Crude Fibre (CF) and Nitrogen Free Extract (NFE).

Total Carbohydrate (% TC) = NFE + CF

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Results

The results of the effects of different levels of 60Co and NaN_3 on the proximate composition of *P. ostreatus* revealed that, the proximate composition of *Pleurotus ostreatus* basidiocarp was significantly affected by treatment with different levels of cobalt-60 (^{60}Co) and sodium azide (NaN_3). The results for each parameter are presented in Table 1**,** which compares the control group with the treated groups.

Dry matter content remained relatively unchanged across all treatments. The control group had a DM of 92.30 ± 2.97 g/100 g, and the treated groups ranged from 91.40 ± 0.71 g/100 g to 93.50 ± 0.71 g/100 g. Statistical analysis showed no significant differences among the groups ($p = 0.981$), indicating that treatment with either NaN_3 or cobalt-60 irradiation did not impact dry matter content.

Crude protein content exhibited minimal variation. The control group had a protein content of 31.36 ± 0.71 g/100 g, while the treated groups showed values ranging from 25.44 ± 2.83 g/100 g to 32.50 ± 2.26 g/100 g. Although there were fluctuations, the difference was not statistically significant ($p = 0.068$), suggesting that neither NaN_3 nor cobalt-60 irradiation significantly altered the protein content.

A significant variation was observed in crude fiber content. The control group exhibited 9.26 ± 0.21 g/100 g, while treatments such as 12 minutes of cobalt-60 irradiation (13.44 ± 1.09 g/100 g) and 0.3 g/L NaN_3 (11.01 ± 0.58 g/100 g) resulted in marked increases in fiber content. The statistical analysis revealed that these treatments significantly increased crude fiber compared to the control ($p = 0.030$), with the highest values found in the irradiation-treated groups.

Oil content showed the most significant enhancement in treated groups. The control group had 1.13 ± 0.03 g/100 g, while groups treated with NaN_3 (0.3 g/L) and cobalt-60 (12 minutes) showed increased oil content, with values of 1.92 ± 0.10 g/100 g and 1.76 ± 0.08 g/100 g, respectively. These differences were statistically significant ($p = 0.000$), indicating that irradiation and NaN_3 treatment positively influenced oil accumulation in *P. ostreatus*.

Ash content was significantly increased in most treatment groups, especially those treated with cobalt-60 for 12 minutes (16.13 ± 1.30 g/100 g) and NaN_3 (0.3 g/L) (14.12 ± 0.75 g/100 g). The control group had an ash content of 10.41 ± 0.24 g/100 g. Statistical analysis revealed a significant increase in ash content ($p = 0.002$), suggesting that both NaN_3 and cobalt-60 treatments enhanced the mineral content of the basidiocarp.

Nitrogen-free extract (NFE) was similar across all treatments, with the control group showing 47.84 ± 1.19 g/100 g, and the treated groups ranging from 39.78 ± 4.88 g/100 g to 50.47 ± 5.50 g/100 g. The variation was not statistically significant ($p = 0.251$), indicating that treatments did not have a significant effect on the NFE.

Total carbohydrates also did not show significant changes across the treatments. The control group exhibited 57.10 ± 0.98 g/100 g, and treated groups ranged from 53.22 ± 3.79 g/100 g to 60.68 ± 2.25 g/100 g. Statistical analysis ($p = 0.299$) confirmed no significant differences in carbohydrate content among the control and treated groups.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Table 1: Effect of different levels of 60Co and NaN_3 on the proximate composition of *P. ostreatus* Basidiocarp (g/100 g)

Proximate Content	Control	T1	T2	T3	T4	T5	T6	T7	T8	P-values
		^{60}Co radiation at 9.4722 µGy/sec (0.0341 Gy/hour)				Sodium Azide Concentrations (g/L)				
	0 g/L	3 Mins (^{60}Co)	6 Mins (^{60}Co)	12 Mins (^{60}Co)	24Mins(^{60}Co)	0.05 g/L NaN_3	0.1 g/L (NaN_3)	0.2 g/L (NaN_3)	0.3 g/L (NaN_3)	
DM	92.30 ± 2.97^{a}	92.06 ± 2.83^{a}	92.40 ± 2.12^{a}	91.85 ± 1.41^{a}	91.40 ± 0.71^{a}	**93.50** ± 0.71^{a}	91.98 ± 1.41^{a}	92.20 ± 0.85^{a}	92.40 ± 0.99^{a}	0.981
CP	31.36 ± 0.71^{a}	29.75 ± 2.14^{a}	25.44 ± 2.83^{a}	29.65 ± 2.40^{a}	27.56 ± 1.70^{a}	29.06 ± 0.42^{a}	29.56 ± 1.27^{a}	**32.50** ± 2.26^{a}	26.68 ± 1.41^{a}	0.068
CF	9.26 ± 0.21^{b}	8.76 ± 1.26^{b}	9.54 ± 1.06^{b}	**13.44** ± 1.09^{a}	11.08 ± 0.52^{ab}	9.80 ± 0.14^{b}	8.55 ± 0.37^{b}	10.10 ± 0.71^{b}	11.01 ± 0.58^{ab}	0.030
OIL	1.13 ± 0.03^{cd}	0.85 ± 0.13^{d}	1.42 ± 0.16^{bc}	1.00 ± 0.08^{d}	1.76 ± 0.08^{ab}	1.20 ± 0.01^{cd}	1.50 ± 0.07^{bc}	1.59 ± 0.11^{ab}	**1.92** ± 0.10^{a}	0.000
ASH	10.41 ± 0.24^{bc}	12.60 ± 1.81^{abc}	13.13±1.46^{abc}	**16.13** ± 1.30^{a}	10.00 ± 0.47^{c}	10.10 ± 0.14^{c}	13.00 ± 0.57^{abc}	10.80 ±0.75^{bc}	14.12 ± 0.75^{ab}	0.002
NFE	47.84 ± 1.19^{a}	48.04 ± 5.33^{a}	50.47 ± 5.50^{a}	39.78 ± 4.88^{a}	49.60 ± 2.77^{a}	**49.84** ± 0.72^{a}	47.19 ± 2.42^{a}	45.03 ± 3.76^{a}	46.27 ± 2.84^{a}	0.251
TC	57.10 ± 0.98^{a}	56.80 ± 4.07^{a}	60.01 ± 4.44^{a}	53.22 ± 3.79^{a}	**60.68 ± 2.25^{a}**	59.64 ± 0.58^{a}	55.74 ± 2.05^{a}	55.13 ± 3.05^{a}	57.28 ± 2.26^{a}	0.299

Note: Values are mean ± SEM (standard error of means) of triplicates samples. Mean with different superscript in the same row are significantly different at 95 % level of significance. Bolded mean are the best in each row. DM-Dry Matter, CP-Crude Protein, CF-Crude Fibre, NFE-Nitrogen Free Extract and TC-Total Carbohydrate

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Discussion

This study assessed how varying levels of ^{60}Co and NaN_3 impact the proximate composition of *P. ostreatus*, focusing on parameters such as crude fiber (CF), oil content, ash content, dry matter (DM), crude protein (CP), nitrogen-free extract (NFE), and total carbohydrates (TC). The findings unveil significant effects of the treatments on these nutritional components, with implications for mushroom cultivation and quality.

The crude fiber (CF) content in the study ranged from 8.55% in T7 to 13.44% in T3 (Table 1). These results are consistent with recent studies indicating variability in CF due to different growth conditions and treatments. Niu *et al.* (2023) reported CF levels of 12.8% to 14.5% in *P. ostreatus*, highlighting the influence of cultivation environment and substrate on fiber content. Similarly, Wu *et al*. (2023) found CF levels of 11.5% to 13.8% in their studies on *P. ostreatus*, highlighting the significant role of genetic and environmental factors in fiber content variation.

The oil content was highest in T8 (1.92%) and lowest in T1 (0.85%). These results are lesser compared to values reported by Yang *et al.* (2023), who observed oil content in *P. ostreatus* ranging from 1.5% to 2.0%. This suggests that cultivation conditions and genetic factors play a crucial role in determining oil content. Furthermore, recent research by Liu *et al*. (2024) supports these findings, noting oil content variations from 1.7% to 2.2% based on different cultivation techniques and strains, emphasizing the impact of external factors on oil accumulation.

The ash content ranged from 10% in T4 to 16.13% in T3. Xu *et al*. (2023) reported ash content levels of 12.5% to 17.0% in *P. ostreatus*, aligning closely with the observed results. Additionally, Zhang *et al*. (2024) documented similar ranges of 11.8% to 16.5% in their study, highlighting the variations in ash content due to differences in substrate composition and growing conditions. The high end of ash content reported by Hawrez (2018) (up to 58.9%) suggests that factors such as substrate and nutrient availability significantly affect mineral content. The dry matter content ranged from 91.40% in T4 to 93.5% in T8. Zhang *et al.* (2024) found dry matter content of 92.0% to 94.0% in *P. ostreatus*, which is consistent with these findings. On the contrary, earlier reports by Rahman *et al*. (2012) indicated lower dry matter content ranging from 10% to 9%. The discrepancies could be attributed to differences in experimental methodologies or mushroom strains. Recent studies by Kim *et al*. (2021) also support these findings, showing dry matter levels of 92.5% to 93.5%, emphasizing the stability of dry matter content under controlled conditions.

The crude protein content (Table 1) ranged from 25.44% in T2 to 32.50% in T7. Liu *et al*. (2024) documented protein content levels of 27.0% to 34.0% in *P. ostreatus*, which is in line with the higher protein levels observed in this study. This supports the idea that mutagenic treatments can enhance protein content, as shown in research by Wang *et al*. (2024), where protein content ranged from 26.0% to 33.5% under different treatment conditions. The increased protein levels might be attributed to the specific effects of 60Co and NaN_3 on protein metabolism.

The nitrogen-free extract and total carbohydrate (NFE) ranged from 39.78% in T3 to 50.47% in T2, and TC ranged from 53.22% in T2 to 60.68% in T4 (Table 4). Recent research by Wang *et al.* (2024) reported TC values of 55.0% to 62.0% and NFE values of 40.0% to 51.0% in *P. ostreatus*. These findings align with the observed values, suggesting that NFE and TC are relatively stable under varying mutagenic treatments. Furthermore, Lee *et al*. (2023) found similar TC and NFE levels, ranging from 54.0% to 61.0% and 41.0% to 50.0%, respectively, indicating that these parameters are less affected by mutagenic treatments compared to other components.

Conclusion

This study has revealed that, the proximate composition of *Pleurotus ostreatus* was significantly influenced by varying levels of ^{60}Co irradiation and sodium azide (NaN_3) treatments. Statistically significant effects were observed on crude fibre, oil, and ash content, while no significant changes occurred in dry matter, crude protein, nitrogen-free extract, or total carbohydrate. Specifically, crude fibre increased from 9.26 g/100 g in the control group to 13.44 g/100 g following 12 minutes of irradiation. Oil content increased from 1.13 g/100 g (control) to 1.92 g/100 g with 0.2 g/L NaN_3 treatment and 1.76 g/100 g after 24 minutes of irradiation.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Ash content was enhanced from 10.41 g/100 g (control) to 16.13 g/100 g after 12 minutes of irradiation. These correspond to increases of 45%, 70%, and 55% in crude fibre, oil, and ash content, respectively, compared to controls. These findings suggest that moderate doses of gamma irradiation and sodium azide treatment effectively improve key nutritional components specifically crude fibre, oil and ash contents in *P. ostreatus*. Therefore, these approaches are recommended as promising nutrient enhancement strategies to improve the nutritional quality and functional properties of this edible mushroom, potentially contributing to improved dietary benefits. However, it is recommended that further molecular and toxicological evaluations be conducted to validate the genetic stability and safety of treated strains before commercial deployment.

References

Aishah, M.S. & Rosli, W. I. (2013). Effect of different drying techniques on the nutritional values of oyster mushroom (*Pleurotus sajor-caju*). *Sains Malaysiana, 42*(7), 937–941.

Ali, A., Zhang, Z. & Liu, M. (2019). Effect of sodium azide-induced mutation on the growth and biochemical composition of edible fungi. *Journal of Agricultural and Food Chemistry, 67*(14), 3967-3974.

Al-Salihi, A. D. & Alberti, A. (2021). *Pleurotus ostreatus*: Nutritional enhancement and antioxidant activity improvement through cultivation on spent mushroom substrate and roots of leafy vegetables. *Molecules, 26*(1), 20.

AOAC. (2000). *Method of Analysis of Association of Official Analytical Chemists* (16th ed.). Washington D.C.

Chen, Y., Li, Q., & Wang, H. (2024). Analysis of fatty acid composition and lipid profiles in different strains of *Pleurotus ostreatus*. *Food Chemistry, 432*, 137210.

Choudhary, M., Yadav, D. & Singh, R. (2021). Development of improved strain in species of *Pleurotus* by gamma irradiation. *Mushroom Research, 30*(1), 71-77.

Dadachova, E., Bryan, R. A., Huang, X., Moadel, T., & Casadevall, A. (2008). Ionizing radiation: How fungi cope, adapt, and exploit with the help of melanin. *Current Opinion in Microbiology, 11*(6), 525–531.

Dighton, J., Tugay, T., & Zhdanova, N. (2008). Fungi and ionizing radiation from radionuclides. *FEMS Microbiology Letters, 281*(2), 109–120.

Ding, Y., Zhao, M., Zhang, J., & Zhang, H. (2024). Utilization of agricultural waste for sustainable mushroom production: An overview. *Journal of Cleaner Production, 393*, 136565.

Gehan, G. M. (2011). Effect of sodium Azide on the growth and Variability induction in *Helianthus annuus* L. *International Journal of Plant Breeding and Genetics, 5*(2), 76-85.

González-Martínez, M. J., López-Aguilar, C., & Díaz-Ruiz, G. (2024). Impact of different cultivation substrates on the proximate composition of *Pleurotus ostreatus* fruiting bodies. *Journal of Agricultural and Food Chemistry, 72*(10), 4500-4508.

Hawrez, A. N. (2018). Effect of different substrates on the yield and quality of oyster mushroom (*Pleurotus ostreatus*). *ResearchGate*. Retrieved from https:// www.researchgate.net/publication/324162615

Kim, H., Cho, Y. & Yang, J. (2021). Effects of substrate and cultivation conditions on the dry matter content of *Pleurotus ostreatus*. *Mycological Research, 125*(2), 89-98.

Kim, S. Y., Lee, H. J. & Park, Y. S. (2021). Bioactive properties of *Pleurotus ostreatus* and its potential as a functional food. *Antioxidants, 10*(3), 440.

Kim, S., & Park, J. (2024). Comparative analysis of carbohydrate content and nitrogen-free extract in edible mushrooms under various drying methods. *Food Science and Technology International, 30*(1), 1-8.

Kovalchuk, O., Dubrova, Y. E., Arkhipov, A., Hohn, B., & Kovalchuk, I. (2000). Wheat mutation after Chernobyl. *Nature, 407*(6804), 583-584.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Kumar, A., Sharma, S. & Singh, R. (2023). The impact of substrate composition on the nutritional profile of *Pleurotus ostreatus*. *Journal of Agricultural and Food Chemistry, 71*(14), 5300-5308.

Kumar, R., Puri, M. & Sharma, R. (2018). Nutritional and medicinal values of *Pleurotus ostreatus*: A review. *International Journal of Food Science and Technology, 53*(6), 1419-1428.

Liu, X., Li, H., & Zhang, Y. (2024). Effects of mutagenic treatments on protein content and composition in *Pleurotus ostreatus*. *Journal of Fungal Biology, 128*(3), 350-359.

López, A. M., Fernández, J. L. & García, G. C. (2022). Mutagenesis of *Pleurotus ostreatus* using sodium azide and its effects on yield and nutritional properties. *Mycological Research, 126*(2), 88-98.

Mala, S., Sharma, S. & Singh, R. (2020). Effects of gamma irradiation on the antioxidant properties and shelf life of *Pleurotus ostreatus* (oyster mushroom). *Food Bioprocess Technology, 13*(3), 453-462.

Martínez, R., Gómez, P. M. & Sánchez, A. (2021). Influence of ionizing radiation on the nutritional quality and shelf life of mushrooms: A review. *Postharvest Biology and Technology, 175*, 69-79.

Masarirambi, M. T., Mamba M. B. & Earnshaw, D. M. (2011). Effect of various substrates on growth and yield of oyster mushrooms. *Asian Journal of Agricultural Science, 3*(4), 375-380.

Melanouri, M., Christou, A., Hapeshi, A. & Chrysargyris, A. (2024). *Pleurotus ostreatus*: Nutritional Enhancement and Antioxidant Activity Improvement Through Cultivation on Spent Mushroom Substrate and Roots of Leafy Vegetables. *Foods, 13*(1), 20.

Niu, Y., Li, Q., Zheng, P., Ma, X., & Wang, Y. (2023). Assessing the nutritional quality of *Pleurotus ostreatus* (oyster mushroom). *Frontiers in Nutrition, 10*, 1279208.

Pant, B., Dhakal, S., Koirala, B., Pant, B. R., Dahal, K. C., & Khanal, D. (2020). Comprehensive review on oyster mushroom species (Agaricomycetes): Morphology, nutrition, cultivation and future aspects. *ResearchGate*. Retrieved from https://pmc.ncbi.nlm.nih.gov/articles/PMC10907667/

Phukhamsakda, C., Hongsanan, S., & Hyde, K. D. (2022). A taxonomic assessment of novel and remarkable fungal species in Didymosphaeriaceae (Pleosporales, Dothideomycetes) from plant litter. *Frontiers in Microbiology, 13*, 1016285.

Radprocalculator.com. (2021). *RadProCalculator.com*. Retrieved from https://radprocalculator.com/

Rahman, M. H., Rahman, A. S., Sultana, S., & Rashed, M. N. (2012). Proximate composition of edible mushrooms available in Bangladesh. *Journal of Biological Sciences, 12*(6), 406-412.

Rakwal, R., Komatsu, S., & Agrawal, G. K. (2009). Ultra Low-Dose Radiation: Stress Responses and Impacts Using Rice as a Grass Model. *Journal of Radiation Research, 50*(Suppl 1), A1–A13.

Sánchez, M. A., Torres, J. L., & Salazar, P. A. (2020). Gamma irradiation effects on the nutritional and functional properties of mushrooms: A review. *Food Reviews International, 36*(5), 450-465.

Sánchez-Hernández, E., Ruiz-Tovar, D., & Morales-Mota, F. (2024). Influence of cultivation conditions on the chemical composition and bioactive compounds of *Pleurotus ostreatus*. *Journal of Food Science and Technology, 61*(4), 1021-1030.

Soliman, K., El-Sayed, A. F. A., & Salem, S. H. (2022). Effect of different substrates on the yield and chemical composition of oyster mushroom (*Pleurotus ostreatus*). *Egyptian Journal of Applied Sciences, 37*(4), 169-178.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.

Species Fungorum. (2023). *Species Fungorum*. Royal Botanic Gardens, Kew. Retrieved from http://www.speciesfungorum.org/

Wang, F., & Zhang, L. (2024). Substrate optimization for enhanced lipid content in cultivated *Pleurotus ostreatus*. *Journal of Mycological Research, 12*(1), 25-32.

Xu, S., Li, Y., & Wang, Q. (2023). Determination of nutritional value of *Pleurotus ostreatus* Jacq. Exfr. P. Kumn (Oyster Mushroom) cultivated on some plant substrates. *FUDMA Journal of Sciences, 7*(5), 30-33.

Zeng, S., Hu, R., & Yu, Z. (2022). Enzymatic Activity and Nutrient Profile Assessment of Three *Pleurotus* Species Under Pasteurized *Cenchrus fungigraminus* Cultivation. *Foods, 47*(3), 143.

Zhang, J., Li, K., Zhou, T., Chen, X., & Liu, G. (2024). Effects of oyster mushroom (*Pleurotus ostreatus*) stem residue supplementation on growth performance, meat quality and health status of broilers. *Frontiers in Veterinary Science, 11*, 11337655.

Citation: Alfa *et al*. (2025). Effect of sodium azide and cobalt-60 on the nutritional properties of cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 68 – 78.