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Preliminary Shelf Life Studies of *In-vitro* Antioxidant Potential of Gamma Irradiated Dried Mushrooms (*Pleurotus ostreatus* Ex. Fries) Kummer in Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NKK, GTO and VA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MO, VA and DLNM managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors RMA, PTA and MWK managed the experimental process and authors MO and GTO identified the species of mushrooms. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Antioxidant potential of dried and gamma irradiated mushrooms (*Pleurotus ostreatus*) were assessed over a 12 months storage period.
Place and Duration of Study: Laboratory work was carried out at the Applied Radiation and Biological Centre, Radiological and Medical Research Institute, Ghana Atomic Energy

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Commission, Accra. From 25th June 2013 to 25th June 2014.
Methodology: Mushrooms were stored in polythene and polypropylene packs and were irradiated with ionizing radiation from a cobalt-60 source at doses of 0.5, 1, 1.5, 2 kGy at a dose rate of 1.7 kGy/hr at room temperature of 28-30℃. The control was not irradiated. Total phenolic contents, flavonoids and free radical scavenging activity DPPH (2, 2'-diphenyl-1-picrylhydrazyl) were examined at 0, 3, 6 and 12 months of storage using aqueous, ethanol and methanol extracts by Folin-Ciocalteu's method.
Results: Total phenolic content ranged from 0.50±0.01- 10.96±1.7 mg/GAE, flavonoids ranged from 1.64±0.05- 8.90±0.6 mg/QE, DPPH radical scavenging activity also ranged from 6.85±0.10-13.03±0.04% and IC_{50} values also ranged from 0.069 - 1.071 mg/ml. Both extracts and the treatment doses of mushrooms stored in polythene and polypropylene packs differed significantly ($P<0.05$). Good linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of mushroom extracts.
Conclusion: *Pleurotus ostreatus* could be regarded as a promising candidate for natural mushroom sources of antioxidants with high value due to the presence of high phenolic compounds which contribute to high antioxidant activity. Employing low dose gamma radiation by the local food industry could enhance the hygienic quality, extend shelf-life, and preserve nutrients and anti-nutrients.

Keywords: Antioxidant; gamma radiation; Pleurotus ostreatus; total phenolic content; flavonoids; IC_{50}.

1. INTRODUCTION

Mushrooms have been used for many years as nutritional food and food flavouring materials as well as medicines [1-3]. Because of their flavour and aroma, mushrooms are greatly appreciated in many countries. In Ghana, several mushroom species are collected from the wild in forest zones during rainy seasons [4,5] and used for diverse purposes for thousands of years [6] an activity which dates back the emergence of ethnomycology as a discipline in many parts of the world. The use of familiar mushroom species such as *Termitomyces robusta, Termitomyces globulus, Coprinus disseminatus, Volvariella volvacea* (Domo in Akan language) and *Pleurotus* spp. as blood tonic, treatment for blood pressure in hypertensives, etc are well documented by some researchers [7-9]. Nonetheless, the consumption of some species of mushrooms not properly identified and documented, causes minimal exploitation of their full potential and results in the mushrooms remaining mysterious. A lot of mushrooms have had their potency either exaggerated or underutilized. Some special mushrooms species according to oral folklore [10] has had attention to date due to speculative peculiar attributes such as natural sex enhancing boosters for men, rheumatism treatment, purgative drugs and hallucinative effects etc. when consumed while others unfortunately, have become extinct as a result of human activities during farming (weedicide, fungicides, fertilizer etc. application), urbanization and annual wild fire outbreak.

The poor identification and documentation as outlined by [11] of edible and medicinal species of mushrooms in many developing nations including Ghana over the years have created some degrees of inconsistencies in their usages relative to folk medicine practice, food and mythological beliefs. Their relevance in modern day pharmaceutics and nutraceuticals is a product of human experimentation over time. Factors that may be anthropogenic, ethnographic, ethnoecological/ environmental have been implicated in mushrooms underutilization and under-exploration of mushrooms in West Africa.

These observations still form the basis of modern scientific studies of fungal medicinal activities, especially in the field of cancer development. It is therefore essential and noteworthy how reliable these facts collected by traditional West African medicine are in the study of medicinal mushrooms [12-14].

Polyphenols are secondary metabolites distributed moderately in fungi which have important primary roles as defense against plant and mushroom pathogens and fungivore aggression or mycophagy and as response to various strenuous abiotic conditions, such as drought and ultraviolet or gamma radiation [15]. According to Lima et al. [16], some synthetic phenolic antioxidants such as Butylatedhydroxyanisol (BHA), Tert-butylhydroquinone (TBHQ), Butylatedhydroxytoluene (BHT), 2-Tert-butyl-4-methylphenol (TBMP) and gallic acid

esters are currently used in food industry and are considered harmful to human health. Studies by some researchers [17,18] have shown that high doses of TBHQ exert negative health effects on laboratory animals, such as DNA damage, which may lead to stomach tumors. BHA has also been reported to have the same genotoxic and carcinogenic effects in some animal tissues [19]. The current concern about adverse effects of synthetic antioxidants should be reiterated. Mushrooms as nutraceuticals may represent an alternative source of accessible natural antioxidants and a possible food supplement or exploited for other pharmaceutical applications. Explaining the mechanisms of polyphenols metabolism, it is an essential step in appreciating their health effects *in vivo*. Gut absorption and metabolism of these compounds depend on their connection with microbes of the intestines [20].

A debate has been raised about the role of fungi derived mycochemicals on the gut microflora since food is the main source of intake of natural polyphenols. The gut microbes may be altered in composition, biological activity and the mychochemicals could be converted by the colonic microbiota to bioactive compounds like steroids, terpenoids, β-glucans etc. that can affect host health [21,22]. Polyphenols are widely known to possess certain antimicrobial properties which suggest a development into new food preservatives [23] owing to the increasing consumer demands on the food industry to avoid synthetic preservatives, or to develop innovative therapies for the treatment of various microbial infections [24,25] considering the increase in microbial resistance against predictable antibiotic therapy [26].

Sequences of chemical reactions results in an imbalance between oxidant and antioxidant reactions and is typically referred to as oxidative stress [27,28]. Both classes of substances (oxidants and antioxidants) are generated in an oxidation- reduction (redox) set-up, where oxidations involve electron loss and reduction, electron gain [29,30] and has been implicated as causes of a lot of degenerative diseases such as atherosclerosis, cancer, and tissue damage in rheumatoid arthritis [31,32]. Reactive species are commonly identified as substances leading to the oxidation of lipids (lipoxidation), glucose (glycation) and proteins (carbonylation). The reaction products generated in lipoxidation are represented by malondialdehyde, glyoxal, acrolein, 4-hydroxy-nonenal (HNE). Those generated by glycation are methylglyoxal and glyoxal. These compounds react with proteins and amino acids resulting in amino-glycated end products (AGEs) [33]. Brownlee [34] outlined that AGEs are involved in regular events, which may trigger obesity and insulin resistance, diabetes mellitus II and inflammation, and could correspond to important activators of inflammation in various tissues. These outcomes appear quite encouraging but, on the other hand, most of them resulted from fresh and non pretreated sample materials.

Radiation treatments of biological systems have been employed for various processes: reduction of microbial contamination of food, sterilization of materials and increasing the safety and shelf life of foods [35]. The major constraint when using a gamma irradiated phenolic derivative that proves to possess elevated antioxidant and antimicrobial efficacy concerns its stability when stored for a long period of time. There are several reports on effect of irradiation processing on phenolic and flavonoid content as well as antioxidant activity from several plant and food products. Although, some studies report that, gamma irradiation does maintain or enhance the anti-oxidant properties; there are a few examples wherein the antioxidant properties of the food material were decreased [35].

This paper evaluates the stability of total phenolic content, total flavonoid content and antioxidants in dried mushrooms stored for 12 months period following gamma irradiation.

2. MATERIALS AND METHODS

Analytical ethanol, methanol and sodium hydrogen carbonate NAHCO3 were purchased from Sigma-Aldrich, USA. Standards of phenolic acids (gallic acid [3, 4, 5-Trihydroxybenzoic acid]) and of flavonoids, Potasium Acetate, Quercetin [3, 3',4', 5, 7-Pentahydroxyflavone], and 2,2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. The Folin-Ciocalteu's phenol reagent, and Aluminium chloride (AlCl3) were from FlukaChemie AG, Buchs, Switzerland.

Micropipette 100-1000 µl (Iso lab, Germany), sensitive balance (Sartorius, Germany), ultra sonic bath, electric shaker (Vortex Genie 2, USA), Waring blender, moisture analyzer balance (Precisa, Switzerland), centrifuge (Shanghai surgical instruments factory, China), pH-meter (TOA HM-60S, Japan), spectrophotometer (Jasco V-530, USA) were

used for sample preparation and antioxidant activity determination.

Pleurotus ostreatus originally from Mauritius, was cultivated on *Triplochiton scleroxylon* sawdust composted for 28 days, supplemented with 1% $CaCO_3$ and 10% wheat bran [36] at the Mushroom Unit of Food Research Institute-C.S.I.R, Accra, Ghana. Cultivation and harvesting was from the period between September and December, 2012. Mushroom fruit bodies were solar-dried at temperature range of 20- 40 πC to a moisture content of about 12%. Dried mushroom parts were cut up to average size of 2 cm x 3 cm, stored in tight-sealed polythene and polypropylene containers at room temperature. Samples for analysis were ground to powder and stored until needed.

2.1 Irradiation of Mushroom Materials

Forty (40) grams of dried mushrooms were packed into the various containers and irradiated at doses of 0, 0.5, 1, 1.5 and 2 kGy at a dose rate of 1.7 kGyhr -1 from a cobalt 60 source (SLL- 515, Hungary) with an average dose distribution ratio of0.69 in air at the Radiation Technology Centre (R.T.C) of the Ghana Atomic Energy Commission (G.A.E.C). The absorbed dose was determined by using Lithium fluoride photo-fluorescent film dosimeter (SUNNA Dosimeter System, UK).

2.2 Preparation of Mushroom Extracts

Methanolic, de-ionised water (aqueous) and ethanolic extracts of mushrooms were prepared by mixing 10% powder in solvent by constant agitation on a shaker (150 rpm, 30℃, 24 h) [37,38]. Extracts were filtered through Whatman filter paper (No. 1) and the filtrate was centrifuged (10000 rpm, 10℃, 10 min) to obtain a clear supernatant. Its yield was determined and 10 mg/ml stock solution prepared, which was stored in amber coloured bottles at 4℃ till further studies.

2.3 Determination of Total Phenolic Contents in the Mushroom Extracts

The concentration of phenolics in mushroom extracts was determined using spectrophotometric method [39]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 50 ml of methanolic solution of extracts, 2.5 ml of 10% Folin-Ciocalteu's reagent (v/v) dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 50 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45 πC for 45 min. The absorbance was determined using spectrophotometer at λmax = 760 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (mg ml^{-1}) from the calibration line; then the content of phenolics in extracts was expressed in terms of mg of gallic acid equivalent per gram of extract (mg of GAE/g of extract).

Concentration (mg/GAE) = Concentration x Volume /Weight of sample (1)

2.4 Determination of Flavonoid Concentrations in the Mushroom Extracts

The content of flavonoids in the examined mushroom extracts was determined using a modified spectrophotometric method [40]. The sample contained 500 ml of ethanol solution of the extract in the concentration of 1 mg/ml and 100 ml of 10% $AlCl_3$ solution dissolved in 1500 ml ethanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of 500 ml of Quercetin, 100 ml of Potassium acetate (10%) and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of Quercetin equivalent (mg of QE/g of extract).

2.5 Evaluation of Antioxidant Activity and Ic_{50}

The ability of the mushroom extract to scavenge DPPH free radicals was assessed by the standard method [41], adopted with suitable modifications [42]. The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg ml^{-1}. Dilutions were made to obtain concentrations of 0.01, 0.05, 0.1, 0.15

and 0.2 mg ml^{-1}. Diluted solutions of sample (200 ml each) were mixed with 3800 ml of methanolic solution of DPPH. After 30 min incubation in darkness at room temperature (23πC), the absorbance was recorded at 517 nm. Control sample contained all the reagents except the extract. Percentage inhibition was calculated using equation (2). The data were presented as mean values ± standard deviation (n =2).

$$\% \text{ inhibition} = (A_c - A_s)/A_c \times 100 \quad (2)$$

A_c and A_s are absorbance readings for the control and test sample respectively.

IC_{50} value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated by plotting a graph of concentration and % inhibition. A linear regression was estimated to plot x-y and fit data with a straight line. From linear equation y= mx+ c.

Where y= 50 and x calculated.

2.6 Statistical Analysis

Duplicate experimental measurements were carried out and expressed as average of two analyses ± standard deviation. The degree of correlation between variables was done using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, version XIV, 2004). Significances of differences were conducted with a Duncan's Multiple Range Test (DMRT). Linear regression analysis was used to determine total phenolics, total flavonoid contents and antioxidant activity using using Excel for Microsoft Windows 2003.

3. RESULTS AND DISCUSSION

The total phenolic content of the various extracts was compiled in Table 1. For 0- 3 months of storage, aqueous ranged 1.96±0.05 - 10.96±1.7 mgGAE/g. Methanol and ethanol extracts ranged from 0.56± 0.01- 4.81±0.4 mgGAE/g and 1.32 ± 0.02- 2.36±0.2 mgGAE/g respectively.

Generally, fairly superior values of antioxidant activity were recorded. However, there was a general decrease in total phenolic contents in all the extracts which caused some significant ($P<0.05$) differences. Periods of 6-12 months resulted in ranges of 1.90±0.05- 10.60±1.65 mgGAE/g, 1.20-2.38 mgGAE/g and 0.50- 4.81 mgGAE/g for aqueous, ethanol and methanol respectively. There was an apparent effect of 1 kGy irradiation dose generating greater quantity of TPC of 10.60- 10.96 mgGAE/g (Table 1). Irradiation dose of 0.5 kGy produced the most amounts of phenols for all the extracts while 2 kGy recorded least for the extracts.

The increase in phenolic compounds by gamma radiation could be attributed to their release from glycosidic components and the degradation of larger phenolic compounds into smaller ones or significant activation of phenol producing enzymes by gamma irradiation [43]. Conversely, an increase in the radiation dose 1 to 2 kGy did not result in a corresponding increase in the phenolic compounds but rather a decrease in the phenolic compound (Table 1). These results may indicate degradation or insolubilization of phenolic compounds when they are exposed to gamma irradiation [44]. However, studies need to be conducted with more samples to confirm the results.

Comparatively among the extracts, aqueous samples yielded the most of phenols of a range of 1.96-10.96 mgGAE/g (Table 1). Kumari et al. [45] also showed similar results with Triphala, where they reported a significant increase in gallic acid concentration and total phenolics in the water extract due to irradiation that leads to increase in antioxidant property. The phenolic compounds are usually hydrophilic and therefore would be extracted more in aqueous solvents than in methanol. Phenolic compounds such as *p*-Hydroxybenzoic acid, *p*- Coumaric acid and Cinnamic acid have been reported for *P.ostreatus* strain EM 1 [46]. Unekwu et al. [47] recorded significantly ($P<0.05$) higher values of a range of 97.16- 248.80 mgGAE/g for mushrooms species including *P. ostreatus, P. pulmonarius, Auricularia auricula, Hericium erinaceus, Lactarius deliciousus, Cantarellus cibarius, Termitomyces robustus* and *T. manniformis*. In contrast, studies by some researchers [48-50] reported lower values of 0.55 mgGAE/g (*Lentinula edodes*), 8.72 mgGAE/g (*A. fuscosuccinea*), 4.61 mgGAE/g (*A. mesenterica*), 1.04 mgGAE/g (*Tremella fuciformis*) which were within the range of results obtained in this work.
Also, results obtained compares well with common fruits and vegetables noted for their relatively high phenolic constituents such as apple (29.63±0.64 mg/g), banana (9.04±0.32 mg/g), lemon (8.19±0.35 mg/g), orange (8.12±0.11 mg/g), pineapple (9.43±0.15 mg/g), strawberry (16.00±0.12 mg/g), pear (7.06±0.16 mg/g), and grape (4.96±0.26 mg/g) [51,52].

Studies by some researchers [53,54] show that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g ingested daily from a diet rich in mushrooms, fruits and/or vegetables. With regards to packaging material appropriateness, there was no significant (P>0.05) difference between polythene and polypropylene.

The total flavonoid content of the various extracts was compiled in Table 2. Flavonoid contents of doses and solvent extracts differed significantly ($p < 0.05$). Post irradiation storage studies of 0-3 months recorded a range of 3.09±0.2-8.92±0.6, 1.64±0.05- 7.86±0.4 and 2.10±0.1-5.37±0.5 mgQE/g for aqueous, ethanol and methanol respectively. 6-12 months also recorded ranged

Table 1. Effect of irradiation on total phenolic content of dried mushrooms stored in packaging materials for 12 months period

Time (month)	Package	Dose applied (kGy)	Extracts		
			Aqueous (mgGAE/g)	Ethanol (mgGAE/g)	Methanol (mgGAE/g)
0	Polythene	0	3.84±0.4 c	2.00±0.1 b	2.40±0.1 c
		0.5	5.00±0.3 d	2.36±0.2 bc	2.36±0.2 c
		1	4.63±0.3 c	1.54±0.18 b	4.81±0.4 d
		1.5	2.78±0.1 b	1.32 ± 0.02 a	2.83±0.1 c
		2	1.96±0.05 a	1.32±0.02 a	1.96±0.05 b
	Polypropylene	0	2.54±0.1 b	1.32±0.02 a	1.96±0.05 b
		0.5	4.46±0.3 c	1.96±0.05 bc	1.93±0.02 b
		1	10.96±1.7 e	1.53±0.04 b	1.35±0.03 a
		1.5	4.46±0.8 c	1.89±0.07 b	0.56±0.01 a
		2	2.50±0.2 b	1.76±0.05 b	1.40±0.03 a
3	Polythene	0	3.74±0.3c	1.70±0.05a	2.38±0.1c
		0.5	5.11±0.3d	2.31±0.2c	2.36±0.3c
		1	4.60±0.3c	1.50±0.14a	4.80±0.3d
		1.5	2.75±0.1b	1.31±0.02a	2.83±0.2c
		2	1.94±0.05a	1.30±0.01a	1.92±0.05b
	Polypropylene	0	2.54±0.1b	1.32±0.01a	1.91±0.02b
		0.5	4.42±0.3c	1.93±0.05c	1.93±0.03b
		1	10.90±1.7e	1.50±0.03a	1.32±0.02a
		1.5	4.40±0.6c	1.84±0.05b	0.51±0.02a
		2	2.48±0.2b	1.73±0.03b	1.43±0.03b
6	Polythene	0	3.70±0.30c	1.20±0.01a	2.26±0.2c
		0.5	5.03±0.3cd	2.36±0.3c	2.27±0.2c
		1	4.59±0.40c	1.54±0.13b	4.79±0.3d
		1.5	2.70±0.20b	1.27±0.02a	2.83±0.2c
		2	1.90±0.05a	1.30± 0.01a	1.91±0.05b
	Polypropylene	0	2.57±0.10b	1.32±0.01a	1.96±0.02b
		0.5	4.41±0.30c	1.96±0.05b	1.92±0.02b
		1	9.87±1.60e	1.53±0.03b	1.35±0.02a
		1.5	4.42±0.60c	1.89±0.05bc	0.50±0.01a
		2	2.50±0.20b	1.70 ±0.02bc	1.26±0.02a
12	Polythene	0	3.84±0.40c	2.00±0.30b	2.41±0.1c
		0.5	4.81±0.20cd	2.38±0.4c	2.36±0.4c
		1	4.63±0.30c	1.54±0.03b	4.81±0.3d
		1.5	2.73±0.20b	1.32±0.02a	2.83±0.2c
		2	1.94±0.06a	1.30±0.02a	1.92±0.04b
	Polypropylene	0	2.54±0.20b	1.32±0.01a	1.96±0.05b
		0.5	4.42±0.30c	1.96±0.05b	1.93±0.04b
		1	10.60±1.65e	1.51±0.03b	1.30±0.02a
		1.5	4.46±0.70c	1.81±0.05b	0.46±0.01a
		2	2.44±0.30b	1.73±0.02b	1.22±0.03a

Means with same letters in a column are not significantly (P>0.05) different

3.09-8.75, 1.64-7.86 and 2.00-5.37 mgQE/g for aqueous, ethanol and methanol respectively. There were marginal decrements in flavonoid contents over storage period which caused significant ($p<0.05$) changes in aqueous and ethanolic extracts but caused no significant ($P>0.05$) change in methanolic extracts. The decrease might be attributed to some changes in the molecular conformation as a result of low moisture levels which might have solidified or aggregated to immobilize the flavonoids.

There is reduction of the radical scavenger after exposure to high doses of gamma radiation due to displacement from the B-ring to the 2.3-double bond, pointing to a relatively small reactivity (because of the absence of high density spin on a given site) for these intermediate species [55,56]. The survival of such intermediate species after irradiation apparently does not abolish the antioxidant properties and can maintain the radioprotective effects of flavonoids - A feature already noticed in gamma-irradiated strawberry [35,57]. Our results are in harmony with the presence of stabilized quinones, which have been observed after H-abstraction from the B-ring of flavonoids in radiolytic solutions [58]. Irradiation can cause oxidative damage and impair flavor in food. However, the action of effective and radiostable natural antioxidants may prevent chemical oxidation of biomolecules in irradiated foods. Irradiation dose of 0.5 kGy recorded greatest amounts of flavonoids in general for all the extracts. In terms of packaging material suitability, there was no significant ($P>0.05$) difference between polythene and polypropylene. Mujic et al. [53] reported values ranging from 1.61- 5.04 mgCE/g for *L. edodes, Agrocybe aegerita* and *Hericium erinaceus* which were in agreement with our results. Also, Lin et al. [59] reported comparatively low flavonoid values of 2.18 mg QE/g for white variety of *A. fuscosuccinea*, 0.78 mgQE/g for *Tremella fuciformis* and 0.49 mg QE/g for *A. polytricha*. However, values recorded from this work, were higher than works of Obodai et al. [60] and Iwalokun et al. [61] who reported values ranging between 0.2- 2.03µg of RE/g of dry weight and absence of flavonoid extracts in *Pleurotus* sp. The antioxidant concentration in a food material according to Lele et al. [62] depends upon the time of evaluation, like analyzing immediately after the irradiation treatment or after certain period of time duration.

Table 3. shows the percentage inhibition of each extract (aqueous, ethanolic and methanolic) against their DPPH radical scavenger for dried and gamma irradiated mushrooms which were calculated from calibration curves. The analysis of variance (ANOVA) showed that concentration significantly ($P<0.05$) affected percent inhibition. Dried and gamma irradiated *P.ostreatus* stored at 0-3 months, ranged 7.02±0.10- 12.71±0.02, 7.44±0.16- 11.22±0.15 and 8.79±0.06- 13.03±0.04% mgGAE/g for the respective extracts. Storage months 6 to 12 also recorded ranges 7.02±0.10- 12.71±0.02, 7.44±0.16 – 10.81±0.15 and 8.79±0.06- 13.03±0.04% mgGAE/g. Interaction of gamma radiation and storage period resulted in a general significant ($P<0.05$) decrease in percentage inhibition. The process of reduction of free radicals through the system of hydrogen donation from antioxidant compounds. Free radicals used are synthetic DPPH reacts with an antioxidant compound through the donation of electrons from an antioxidant compound to get a pair of electrons. DPPH radical compound deep purple would fade to yellow if it is reduced by antioxidants into non radical DPPH. When the free electron of DPPH radical has been paired with electrons from traps compounds (antioxidants) it would reduce DPPH to radical (DPPH-H), and form stable compounds are DPP Hydrazine [61]. There were significant ($P<0.05$) differences recorded between the aqueous and ethanol extracts. Methanol extracts however, recorded no significant differences with increasing radiation dose. Packaging did not influence results significantly. Present data cannot adequately elucidate the influence of type of extract on yield and activity of DPPH in *P. ostreatus*. Future studies are required to answer this question.

3.1 Half Maximal Inhibitory Concentration (Ic_{50})

This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half [62]. It is commonly used as a measure of antagonist drug potency in pharmacological research. According to [62], IC_{50} represents the concentration of a drug that is required for 50% inhibition in vitro.

The antioxidant activity with $IC_{50}< 0.01$ mg ml^{-1} value corresponds to good antioxidant activity and are included in the category of powerful antioxidants class [63,64].

Table 4. illustrates the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} mg ml^{-1} of

the extracts were in the order aqueous < methanol <ethanol and corresponded to a range of 0.069- 0.073, 0.22- 0.27 and 1.020- 1.071 mg ml^{-1} respectively over the 12 month storage period. IC_{50} of the standard compound quercetin was 0.00287mg ml^{-1}.

The highest radical scavenging activity was shown by aqueous extract obtained from mushrooms stored for 3 months with IC_{50}= 0.069 mg ml^{-1} which is lower than that of quercetin (P<0.05). All the extracts did not show significant difference (P<0.05) from the standard compound except for ethanol (Table 4).

The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds in mushrooms contribute to the

Table 2. Effect of irradiation on flavonoid content of dried mushrooms stored in packaging materials for 12 months period

Time (month)	Package	Dose applied (kGy)	Extracts		
			Aqueous (mgQE/g)	Ethanol (mgQE/g)	Methanol (mgQE/g)
0	Polythene	0	7.67±0.8c	4.25±0.2b	3.93±0.2c
		0.5	8.92±0.6d	7.27±0.4c	3.81±0.2c
		1	8.07±0.7c	5.58±0.2c	4.61±0.4d
		1.5	3.70±0.2a	1.64±0.05a	3.51±0.2b
		2	6.73±0.5bc	7.88±0.4d	2.88±0.1b
	Polypropylene	0	3.09±0.2a	4.73±0.2b	4.18±0.2d
		0.5	8.75±0.8d	7.86±0.4c	5.37±0.5e
		1	7.92±0.6c	4.42±0.2b	2.50±0.2a
		1.5	7.92±0.6c	6.29±0.4c	2.41±0.1a
		2	3.46±0.3a	5.88±0.3c	4.90±0.4e
3	Polythene	0	7.53±0.7c	4.31±0.2b	3.84±0.2c
		0.5	8.88±0.6c	7.27±0.4c	3.81±0.2c
		1	8.00±0.7c	5.58±0.2b	4.58±0.4d
		1.5	3.74±0.2a	1.64±0.05a	3.72±0.2c
		2	7.03±0.5c	7.81±0.4c	2.88±0.1b
	Polypropylene	0	3.09±0.2a	4.69±0.2b	4.14±0.2d
		0.5	8.75±0.8d	7.86±0.4c	5.37±0.5e
		1	7.92±0.6c	4.42±0.2b	2.50±0.2a
		1.5	7.92±0.6c	6.29±0.4c	2.10±0.1a
		2	3.41±0.3a	6.02±0.3c	5.11±0.4e
6	Polythene	0	7.67±0.8c	4.33±0.2b	3.93±0.2c
		0.5	7.92±0.6c	7.27±0.4c	3.81±0.2c
		1	7.87±0.7c	5.51±0.2b	4.61±0.4d
		1.5	3.69±0.2a	1.69±0.05a	3.51±0.2bc
		2	6.73±0.5c	7.88±0.4d	2.88±0.1b
	Polypropylene	0	3.09±0.2a	4.57±0.2b	4.18±0.2d
		0.5	8.75±0.8d	7.86±0.4d	5.37±0.5e
		1	7.92±0.6c	4.42±0.2b	2.50±0.2a
		1.5	7.88±0.6c	6.35±0.4b	2.00±0.1a
		2	3.46±0.3a	5.78±0.3c	4.93±0.4e
12	Polythene	0	7.63±0.8c	4.25±0.2ab	3.93±0.2cd
		0.5	8.90 ± 0.6d	7.27±0.4c	3.81±0.2c
		1	8.00±0.7c	5.52±0.2c	4.61±0.4d
		1.5	3.7±0.2a	1.64±0.05a	3.51±0.2b
		2	6.73±0.5bc	7.82±0.4d	2.88 ±0.1b
	Polypropylene	0	3.05±0.2a	4.73±0.2b	4.18±0.2cd
		0.5	8.72±0.8c	7.86±0.4d	5.37±0.5a
		1	7.92±0.6c	4.42±0.2b	2.50±0.2a
		1.5	7.89±0.6c	6.29±0.4c	2.00±0.1a
		2	3.46±0.3a	5.81±0.3c	4.90±0.4d

Means with same letters in a column are not significantly (P>0.05) different

Table 3. Percentage inhibition (%) of DPPH in dried and irradiated mushrooms stored in packaging materials for 12 months storage period

Time (month)	Package	Dose applied (kGy)	Extracts Aqueous (%) (mgGAE/g)	Ethanol (%) (mgGAE/g)	Methanol (%) (mgGAE/g)
0	Polythene	0	7.02±0.10a	10.06±0.13bc	10.09±0.26b
		0.5	9.65±0.13bc	10.47±0.24c	13.03±0.04b
		1	11.60±0.05d	9.36±0.12bc	12.82±0.03e
		1.5	9.46±0.12bc	9.36±0.12bc	10.30±0.27b
		2	9.26±0.02b	8.31±0.04a	10.60±0.3b
	Polypropylene	0	9.65±0.13bc	8.95±0.05bc	10.40±0.30b
		0.5	8.24±0.02a	10.81±0.25d	11.21±0.04d
		1	11.12±0.05c	7.44±0.16a	9.09±0.12a
		1.5	12.71±0.02c	8.84±0.05bc	8.79±0.06a
		2	9.37±0.12bc	9.06±0.11bc	10.30±0.3b
3	Polythene	0	6.85±0.11a	9.12±0.05b	10.23±0.25b
		0.5	10.0±0.3b	11.11±0.05d	12.89±0.10e
		1	11.42±0.05c	9.36±0.12b	13.03±0.20e
		1.5	9.04±0.02b	9.27±0.03b	10.00±0.13b
		2	9.15±0.02b	8.30±0.04b	11.32±0.05d
	Polypropylene	0	10.00±0.02b	11.15±0.04de	10.40±0.30b
		0.5	8.02±0.01a	11.22±0.15d	11.21 ± 0.04d
		1	11.51±0.02bc	7.34±0.14a	9.12±0.14a
		1.5	11.60±0.02bc	9.12±0.04b	9.00±0.11a
		2	9.30±0.04b	9.11±0.12b	10.00±0.02b
6	Polythene	0	7.05±0.10a	10.06±0.13bc	10.09±0.26b
		0.5	9.65±0.13bc	10.47±0.24c	13.03±0.04e
		1	11.60±0.05d	9.36±0.12bc	12.82±0.03d
		1.5	9.46±0.12bc	9.36±0.12bc	10.30±0.27b
		2	9.26±0.02b	8.31±0.04a	10.60±0.3b
	Polypropylene	0	9.65±0.13bc	8.95±0.05bc	10.40±0.3b
		0.5	8.24±0.02a	10.81±0.25d	11.21±0.04d
		1	11.12±0.05c	7.44±0.16a	9.09±0.12a
		1.5	12.71±0.02d	8.84±0.05bc	8.79±0.06a
		2	9.37±0.12b	9.06±0.11bc	10.30±0.3b
12	Polythene	0	7.00±0.10a	10.06±0.13bc	10.09±0.26b
		0.5	9.65±0.13bc	10.47±0.24c	13.03±0.04e
		1	11.60±0.05d	9.36±0.12bc	12.82±0.03d
		1.5	9.46±0.12bc	9.36±0.12bc	10.30±0.27c
		2	9.26±0.02b	8.31±0.04a	10.60±0.3b
	Polypropylene	0	9.65±0.13bc	8.95±0.05bc	10.40±0.3c
		0.5	8.24±0.02a	10.81±0.25d	11.21±0.04d
		1	11.12±0.05c	7.44±0.16a	9.09±0.12a
		1.5	12.71±0.02d	8.84±0.05bc	8.79±0.06a
		2	9.37±0.12bc	9.06±0.11bc	10.30±0.3b

Means with same letters in a column are not significantly (P>0.05) different

strong antioxidant activity. Previous studies by Mujic et al. [53] revealed a range of 0.02- 0.198 mg/ml for *L. edodes*, *H. erinaceous* and *A. aegerita* which are comparable to *P. ostreatus*. Qusti et al. [67] also reported comparable values of 0.22, 0.47, 0.54, 0.73 mg/ml for figs, snake cucumber, banana and white grapes respectively which were ranked high in antioxidants.

Although gamma irradiation can damage biomolecules directly by rupturing covalent bonds as a result of transfer of photon energy, and indirectly, by producing free radicals and other non-radical reactive oxygen species [68] its adverse effect was not realized and so was evident in our results (Table 4).

Table 4. Effect of storage time on the half maximal inhibitory concentration (IC_{50}) of dried *P. ostreatus*

	Extracts (mg/ml)			
Time	**Aqueous**	**Ethanol**	**Methanol**	**Quercetin (standard)**
0 month	0.071a	1.038c	0.22a	0.00287a
3 month	0.069a	1.041c	0.25a	0.00287a
6 month	0.073a	1.020c	0.27a	0.00287a
12 month	0.072a	1.071c	0.26a	0.00287a

Means with same letters in a row are not significantly (P>0.05) different

4. CONCLUSION

Our study clearly demonstrates that over the twelve months storage period, gamma irradiation had significant effect on the total phenolic, total flavonoid contents and DPPH radical scavenging activities in the mushroom samples. But packaging materials had no effect. Preservation of these antioxidants is dose dependent, as high doses of gamma radiation has been shown to breakdown some of these compounds. In view of these observations, gamma irradiation could be used to extend shelf life of food products due to its ability to destroy microorganisms, insects and larvae which cause deterioration during storage.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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