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Nutritional Qualities and Shelf Life Extension of Gamma Irradiated Dried *Pleurotus ostreatus* (Jacq. Ex. Fr.) Kummer Preserved in Two Different Storage Packs

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Abstract *Pleurotus ostreatus* has high nutritional value as an important source of protein, carbohydrates, vitamins, mineral elements and is among most favorite mushrooms of the world. Proximate composition and metabolizable energy of these mushrooms were evaluated for their dietary value. Fruit bodies were solar dried to a moisture content of 12% and exposed to low dose ionizing (gamma) radiations of 0, 0.5, 1, 1.5 and 2 kGy at a dose rate of 1.7kGy/hr and stored in polyethylene and polypropylene packs at room temperature (28- 30 ^{o}C) for a period of 12 months. Values ranged 14.11- 15.80%, 6.16- 8.31%, 0.65- 1.24%, 13.56- 15.39%, 12.51- 15.25%, 61.16- 65.50% respectively for moisture, ash, fat, fibre, protein and carbohydrate. Metabolizable energy also ranged 247.8- 284.6 Kcal./100g for 12 months storage. Although there were some significant ($p<0.05$) changes observed for some nutrients due to gamma irradiation and storage, the results obtained showed that the integrity of these nutrients in this mushroom were minimally affected as no adverse effects were observed.

Keywords *Pleurotus ostreatus*, Proximate, Nutritional, Metabolizable Energy, Gamma Irradiation

1. Introduction

Pleurotus ostreatus is a macrofungus which exploit polysaccharides (cellulose and hemicelluloses) usually from a wide range of lignocelluloses to produce expensive protein for human consumption [1]. Their global economic value is now inconceivable, and the reason for the rise in consumption is a combination of their value as food [2, 3] and their medicinal or nutraceutical properties [4–7].Their nutritive as well as medicinal attributes dates back to ancient times as early as 1500 BC culled from ancient literatures.

Mushrooms are highly perishable and deteriorate within a short time after harvest due to its high moisture content and inability to maintain their physiological status.

In Ghana and most parts of the world, drying remains one of the best alternatives of preprocessing this mushroom. Drying of different species of mushrooms have been reported in previous studies [8–11]. It is one of the oldest means of food preservation and is applicable to a wide range of food products including *P.ostreatus* strain EM–1 [12] .The principle behind drying according to Labuza and Altunakar [13] is primarily reduction of moisture to levels low enough to inhibit microbial growth and also slow down enzymatic and other biological reactions that may contribute to food spoilage.

Food irradiation is the intentional exposure of food to ionizing radiation (such as gamma and electron beam) in order to enhance its shelf life without any detrimental effect on food quality as well as the safety of food. After decades of research, development, public debate and consumer acceptance trials in many countries, irradiation has emerged as a safe and viable technology for ensuring the safety and quality of food and for combating food-borne diseases. Indeed it is currently the best available technology according to IAEA, [14] as suitable for treating raw and partially raw food products and those countries which adopt it will benefit greatly in both domestic and international markets.

Perusal of pertinent literature revealed that scanty work had been done in this area of study. The objective of this study was to assess the effect of drying and gamma irradiation on the nutritional quality of mushroom (*Pleurotus ostreatus*) stored in polyethylene and polypropylene packs and stored over a period of 12 months.

2. Material and Methods

2.1. Mushroom Samples

Pleurotus ostreatus strain EM 1 were grown on

composted sawdust as described by Kortei *et al* [15] and harvested at maturity from the cropping house of the Mycology Unit, Food Research Institute- Council for Scientific and Industrial Research, Accra, between the periods of February to May 2013.

2.2. Processing

Drying of Mushroom Samples

Drying was carried out by using a solar dryer at a temperature of 50- 60^{o}C to reduce moisture content to about 12% for an average period of 12 days.

Irradiation of Mushroom Materials

Fruitbodies were solar dried to a moisture content of 12% and exposed to low dose ionizing (gamma) radiations of 0, 0.5, 1, 1.5 and 2 kGy at a dose rate of 1.7kGy/hr in air from a Cobalt 60 source (SLL 515, Hungary) batch irradiator. Doses were confirmed using Fricke's dosimetry system which is a reference chemical dosimeter based on the chemical process of oxidation of ferrous ions (Fe^{2+}) in aqueous sulphuric acid solution to ferric ions by ionizing radiation at the Radiation Technology Centre of the Ghana Atomic Energy Commission, Accra, Ghana.

Storage

Forty (40) grams of dried oyster mushrooms (*Pleurotus ostreatus*) were packed and stored in polyethylene and polypropylene packs at room temperature (28- 30 ^{o}C) for a period of 12 months.

Nutritional Analysis

This study was carried out in the laboratories of Department of Food Science and Nutrition (University of Ghana) from March 2013 to March 2014.

Determination of Moisture:

The moisture content was determined by the gravimetric method of AOAC [16]. Two crucibles were each washed, dried, weighed and 2 g of fresh mushrooms weighed into each of the crucibles. The crucibles were placed in a thermostatically controlled oven (Gallenkamp oven 300 plus series, U.K) and the temperature maintained at 105 ^{o}C for 5 hours, after which they were removed and placed in a dessicator to cool. They were then reweighed. The procedure was repeated until a constant weight was obtained. The moisture content was found by subtracting the final mass from the initial mass.

Determination of Fat Content

Procedure was carried out in accordance with AOAC [16] with modifications. Two grams of dried sample was transferred into a 22 mm x 80 mm paper thimble and a small ball of cotton wool placed in the thimble to prevent loss of sample. A 250 ml round bottom flask was washed and dried at 100 ^{o}C and weighed. Some anti– bumping granules were put into the flask. Fifteen millilitres (15 ml) petroleum spirit of boiling point 60 – 80 ^{o}C was added. A quick fit condenser was connected to a Soxhlet extracter and refluxed for 4 hours at high heat using a heating mantle. The flask was removed and the solvent evaporated on a steam bath. The flask with its contents was put into a dessicator to cool to room temperature. It was then weighed and the mass of fat determined by subtraction.

Determination of Ash Content

Procedure was carried out in accordance with AOAC [16] with modifications. Two crucibles were each washed, dried and weighed and 2 grams of the whole mushrooms weighed into the crucibles. They were then placed in a muffle furnace, pre heated to 600 ^{o}C for 2 hours. The crucibles were then removed, allowed to cool in air, placed in a dessicator to cool completely and re-weighed. The masses of the crucibles and their contents were found by subtraction.

Determination of Total Protein:

According to a modified Buiret method by Burtis & Ashwood [17]. Ten grams of ground mushroom was taken with 50ml of 1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000 × g by a table centrifuge machine (Hardwarefactorystore.com, China). The supernatant was collected and total protein content was measured.

Determination of Crude Fiber:

The method outlined by AOAC [18] was used in this experiment. Half gram (0.5g) of ground dehydrated mushroom was weighed (W1) and passed through a one milliliter (1mm) mesh or screen into 600 ml beaker (without spout). Fifty milliliters (50 ml) cold (room temperature) NDS (the NDF solution) was placed in a beaker on a refluxing unit and allowed to boil. Heat was adjusted to even boiling, keeping the sample particles suspended. Heat was reduced as boiling began, to avoid foaming. It was refluxed for 60 minutes from onset of boiling. Gooch crucible was placed on the filter manifold and rinsed with hot water. The residue was filtered on to the crucible using light suction. It was washed twice with hot water. Mixture was allowed to stand for 5-10 minutes, washed twice with hot water, twice with acetone and dried using suction. For recovery reasons, acetone washing was done separately. The crucible was air dried for 10-15min (some of the acetone will escape) and oven dry for 8 hours or overnight in a forced-air oven at 105°C. It was cooled in a desiccator and weighed (W2) to obtain yield of cell wall. Crucible was ashed at 510°C for 3 hours, allowed to cool and removed from furnace, put in an oven (set at 105°C) cooled and weighed (W3). The loss in weight was the ash free cell wall

$$\%\,\mathrm{NDF} = \frac{W_2 - W_0}{\%\mathrm{DM}} \times 100\%$$

$$\%\mathrm{NDF\ (DMB)} = \frac{\%\mathrm{NDF}}{\%\mathrm{DM}} \times 100\%$$

W_0 = Empty crucible weight.
W_1= Weight of ground sample
W_2 = Weight of sample after cooling in dessicator
W_3= Weight of reweighed sample

Determination of Total Carbohydrate

According to Raghuramalu *et al.,* [19] the content of the available carbohydrate was determined by the following equation [20] :

Carbohydrate (g/100g sample) = [100 – (Moisture + Fat + Protein + Ash + Crude Fiber)]

Determination of Metabolizable Energy Content:

Fat, protein or carbohydrates can supply energy. Metabolizable energy was calculated as described by [16] using the following formula:

ME (Kcal /100g) = [(3.5 X CP) + (8.5 X CF) + (3.5 X NFE)]

Where, ME = Metabolic Energy; CP= % Crude Protein; CF = % Crude Fat; NFE = % Nitrogen Free Extract (carbohydrate)

3. Results and Discussion

3.1. Nutritional Analysis

The results of chemical (nutritional) analysis of gamma irradiated dried mushrooms stored in polythene and polypropylene packs are presented in Figures 1 and 2. The nutritional attributes of edible mushrooms are directly linked to their chemical composition. There is species variation in nutrients but it is dependent on type of substrate, stage of development, environmental conditions and essentially the post-harvest condition of storage [21, 22]. Likewise, the physical status of food (frozen or fresh, solid, liquid or powder) and also its composition influence the reactions induced by radiation [23].

Protein content for 0 month storage ranged from 12.51-15.25%. Post irradiation storage studies revealed a decreasing trend of protein content which was however not significantly different ($p>0.05$) irrespective of ionizing radiation dose and storage package used. After 12 months of storage, protein content ranged from 12.48 - 15.22%. According to Mostafarvi [24], an interaction of ionizing irradiation and proteins could produce chemical reactions depending on the protein structure, state (native or denatured), physical status, amino acid composition, the presence of other substances and the radiation treatment. The most important changes include dissociation, aggregation, cross-linking and oxidation. Arvanitoyannis [23], indicated that low and medium doses induce only a small breakdown of food proteins into lower molecular weight protein parts and amino acids which cause less chemical reactions than steam heat interactions. Protein is an important constituent of dry matter of mushrooms [25, 26]. The digestibility of mushroom protein can be as high as 72 to 83%. Protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms [27]. The average protein content of cultivated edible mushrooms ranges from 3.5-4% of their fresh weight [28].

Protein content range obtained for *P.ostreatus* in this study, generally fell within the range (12.51- 15.25%) reported by several authors [28, 29, 30, 31] who worked on *Pleurotus spp*. [32] Egwim *et al.*, reported low protein content values for *P. ostreatus* (14.03 ± 0.01%) and also investigated some selected wild edible Nigerian mushrooms and recorded higher protein contents of range 26.25±1.93-60.38±0.20% for mushroom species *Cantharella cibarius*, *Laccaria amethysta, Clitocybe odora, Lepista nuda, Macrolepiotata procera, Lepista saeva, Lactarius deliciousus, Laccaria laccata, and Hericium erinaceus*. Al-Momany and Salih, [33] found values of 16.0- 16.8% range for *Pleurotus spp*. amongst other edible fungi such as *Agaricus macrosporus* and *Tricholoma saponaceum* var *squamosum*. In terms of the amount of crude protein, mushrooms rank below animal meats but well above most other foods including milk [34]. On a dry weight basis, mushrooms normally contain 19 to 35% proteins compared to 7.3% in rice, 12.7% in wheat, 38.1% in soybean and 9.4% in corn [35, 27]. A 100g serving can provide about 12.5-15.1% of the recommended dietary allowance (RDA) or recommended nutrient intake (RNI). This high protein content implies that this fungus can contribute significantly to the daily human protein requirements, usually about 23-56g [36, 37].

Mushrooms contain high moisture depending on the mushroom species and other parameters related to harvest, growth, culinary and storage conditions [38]. Moisture content of dried *P.ostreatus* ranged from 14.11- 15.80% for 0-3 month while 6-12 months storage ranged 14.11-16.11% and showed some significant differences ($p<0.05$). This could be attributed to the difference in the extent of water hydrolysis by gamma radiation doses [23]. The moisture content of any food is an index of its water activity [39, 40], and is used as a measure of stability and susceptibility to microbial contamination [41, 42]. Dried mushrooms are not prone to rapid deterioration due to the absence of medium of transport of enzymes to facilitate chemical reactions which cause deterioration.

Although mushrooms are generally low in fat, they do contain essential unsaturated fatty acids and are also cholesterol free and as such considered essential and significant for human diet and health. Fat content ranged from 0.65- 1.24% for 0-3 month while that of 6- 12 months ranged 0.63- 1.24%. The average fat content of mushrooms is reported to be generally low, ranging from 0.6-3.2%. Gamma radiation had a significant ($p<0.05$) effect on the fat content of dried mushrooms during storage. Radiation dose 2 kGy had an apparent significant ($p<0.05$) effect on the fat content. Free radicals formation during irradiation has been proven to increase lipid oxidation [43]. The range of fat

content values obtained (0.65-1.24%) were comparable to the 1.23% – 0.53% reported by Musieba *et al.* [44] and Nurudeen *et al.*[45] respectively.

Crude fibre contents obtained in this present study ranged 13.56- 15.39% and its analysis showed some significant differences ($p<0.05$). Results obtained fell within range of results (3–32%) reported by some researchers [44, 32]. On the average, a 100g serving of mushrooms guarantees from 9 to 40 % of the daily recommendation of dietary fibre [22]. Dietary fibre content was high (approx. 45 % of dry matter). The fairly high level of fibre in the mushroom was a desirable characteristic since fibre plays an important role in human diet [22, 46, 47], observed that glycogen and chitin form the major constituent of fibre content of mushrooms. Gordon [48] indicated that there is a "dietary fibre hypothesis" which suggests that fibre helps to prevent many diseases prevalent in affluent societies. Evidence from epidemiological studies suggest that increased fibre consumption may contribute to a reduction in the incidence of certain diseases like diabetes, coronary heart disease, colon cancer, high blood pressure, obesity, and various digestive disorders [49, 50, 51]. Dietary fibres alter the colonic environment in such a way as to protect against colorectal diseases. It provides protection by increasing faecal bulk, which dilutes the increased colonic bile acid concentrations which occur with a high-fat diet [52].

Carbohydrate values obtained in this study ranged from 61.39- 65.50% which represents the bulk of fruiting bodies accounting for on dry weight basis. Similar results have been reported by other researchers for cultivated *Pleurotus* mushroom [53, 20]. *Pleurotus spp.* dry matter usually include 50- 60% carbohydrates composed of various compounds; monosaccharides, their derivatives and oligosaccharides (commonly called sugars) and both reserve and construction polysaccharides (glycans). Kalac [4], reported a decrease in mannitol and α, trehalose which are the main constituents of oligosaccharides as well as polyols respectively. In general, irradiation modifies mono and polysaccharides, but thermal treatment can produce more modifications [54]. [55] reported carbohydrate values of *Agaricus bisporus* (56.47 ± 0.21 %) and *Agaricus bitorquis* (39.94 ± 0.17 %). The amount of carbohydrates determined in *Agaricus bitorquis* was comparable to results obtained (61.39- 65.50%) as average value for *P.ostreatus* in this present study. Carbohydrate that can be used by humans produces four calories per gram as opposed to nine calories per gram of fat and four per gram of protein. In areas of the world where nutrition is marginal, a high proportion (approximately 0.45- 0.9 kg) of an individual's daily energy requirement may be supplied by carbohydrate, with most of the remainder coming from a variety of fat sources [56].

Ash content values ranged between 6.16- 8.31%. Statistical analysis revealed no significant ($p>0.05$) difference. Results obtained in these present studies were within range of values of 5.69- 7.82% as reported by [57], [58] and [44] respectively. However, [32] recorded higher values of 20.55 ± 0.13 %. By and large, packaging had no significant ($p>0.05$) effect on the proximate analyses.

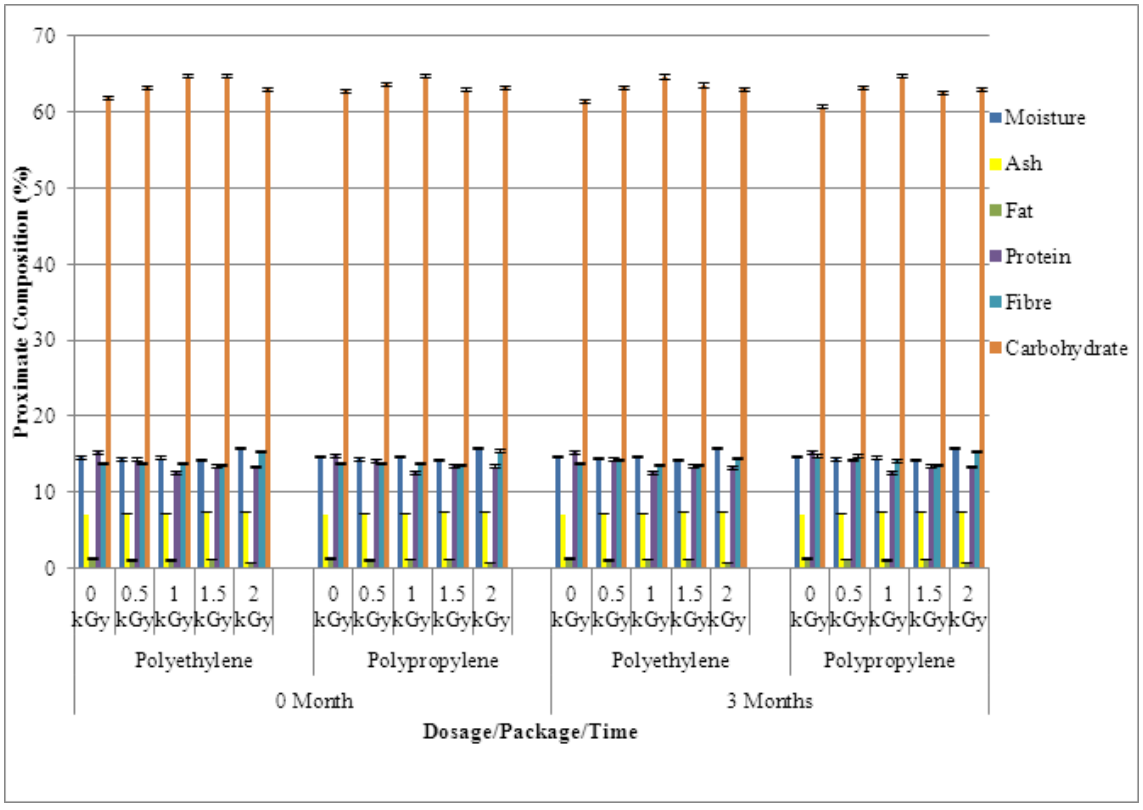

Figure 1. Effect of irradiation on proximate composition of dried mushrooms in storage packs during storage period of 0- 3 months

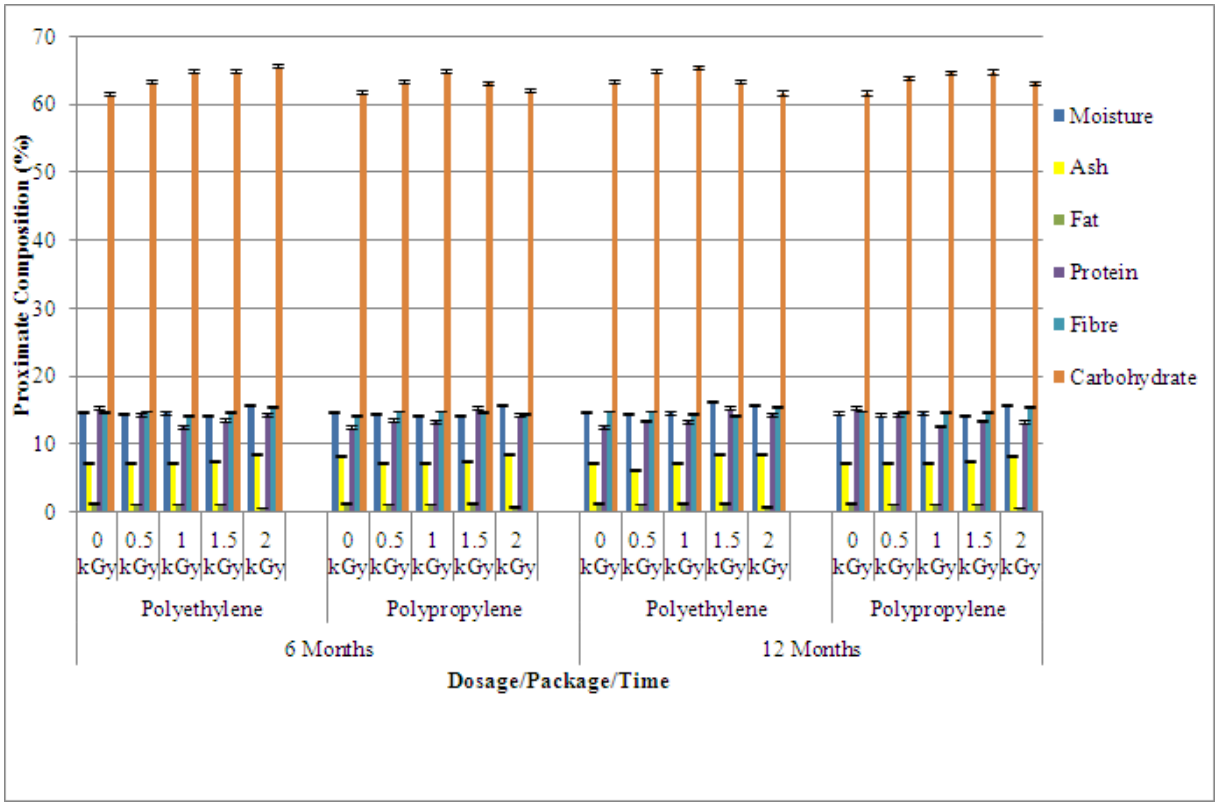

Figure 2. Effect of irradiation on proximate composition of dried mushrooms in storage packs during storage period of 6-12 months

2.2. Metabolizable Energy

Table 3. Effect of irradiation and storage on the Metabolic Energy (Kcal/100g) of Mushrooms

Storage Time (month)	Package material	Applied Dose (kGy)				
		0	0.5	1.0	1.5	2.0
0	P1	280.4^{b}	279.9^{b}	278.9^{b}	283.1^{c}	272.7^{a}
	P2	279.9^{b}	280.8^{b}	279.7^{b}	276.5^{bc}	273.1^{a}
3	P1	273.7^{a}	279.3^{b}	282.1b^{c}	279.5^{c}	276.9^{c}
	P2	274.4^{a}	282.6^{c}	284.6^{c}	275.3^{a}	279.9^{ab}
6	P1	278.6^{b}	281.7^{b}	247.8^{a}	282.9^{b}	283.7^{b}
	P2	279.8^{b}	280.0^{b}	278.7^{b}	277.5^{b}	273.4^{b}
12	P1	280.0^{b}	279.9^{b}	280.0^{b}	284.9^{c}	273.3^{a}
	P2	279.0^{b}	281.8^{b}	278.8^{b}	282.3^{c}	272.0^{a}

Means with same letters in a row are not significantly (P>0.05) different
P1- Polythene, P2- Polypropylene

Generally, energy values of dried and gamma irradiated mushrooms stored in the different packaging materials ranged 247.8- 284.9 Kcal./100g of dried mushrooms and are presented in Table 3. The initial energy values ranged 272.7-283.1 and 273.1- 280.0 Kcal/100g for polythene and polypropylene respectively. There was no significant ($p>0.05$) effect of storage packs on the metabolizable energy. In terms of effect of gamma radiation, dose 2 kGy showed an apparent effect ($p<0.05$) on fat content which affected the energy content indirectly since it constitutes an integral part of the energy equation. After 3 months, energy ranged from 273.7- 282.1 and 274.4- 284.6 Kcal./100g for polythene and polypropylene respectively. There were significant differences ($p<0.05$) in energy values obtained. Storage for 6 months, gave values ranging 247.8-283.7 and 273.4-280 kcal./100g for polythene and polypropylene respectively. In polythene package, irradiation caused significant difference ($p<0.05$). However, polypropylene showed no significant difference ($p>0.05$) to irradiation effect. For storage for 12 month values, ranged from 273.3- 284.9 and 272.0- 282.3 Kcal./100g for both packages respectively. Energy values due to irradiation showed significant differences ($p<0.05$) in both polythene and polypropylene.

[22] emphasized that owing to their high water content and low caloric value, mushrooms could be considered as a dietetic food suitable for low-calorie diets. In terms of energy values, the present results indicated that dry mushrooms stored up to 12 months were of good quality, low in calorie content and their energy value varied significantly ($P<0.05$). Obodai *et al* [58], [6] and [59], reported energy values within range of 272- 389 Kcal./100g and 381- 389 Kcal./100g respectively for *Pleurotus spp*. Mshandete and Cuff [60] reported energy values of 313 Kcal/100g dry matter for *Coprinus cinereus*, 305 Kcal/100g dry matter for *Volvariella volvacea* and 302 Kcal/100 g dry matter *Pleurotus flabellatus*. Egwim *et al* [32] obtained energy values of 305 Kcal/100g and 302 Kcal/100g for *Laccaria amethystea* and *Lepista nuda* respectively. These values fall within what was recorded in the dry mushroom samples stored in both packaging materials before and after irradiation. Recently,

[57] also found energy value of *P.ostreatus* on differently formulated rice straw composts to vary from 305.81- 387.80 Kcal./100g.

4. Conclusions

Our study showed that this mushroom species are good sources of proteins and carbohydrates. Several functional properties have also been detected as favourable, making it potentially useful in many food formulations. This research also demonstrates the ability of gamma irradiation to be used in the preservation of nutritional qualities of foods as changes that occurred due to gamma irradiation were minimal irrespective of the packaging material used in this experiment. Averagely, non-irradiated dried mushroom last not more than 6 months because of insect and pests damage. Additionally, gamma irradiation eliminated all these insects and pests to prolong its shelf life to 12 months. Gamma irradiation with its enormous attributes could be employed in food manufacturing industries to enhance product quality and shelf life.

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