

IRRADIATION OF MUSHROOMS AND ITS IMPACT ON BIOACTIVE COMPONENTS

Dr. Kalyani Bandi¹, Dr. Manjula Kola²

¹Academic Consultant, Division of Food Technology, Department of Home Science, S.V.University, Tirupathi, Andhra Pradesh, India 51750.

²Assistant Professor, Division of Food Technology, Department of Home Science, S.V.University, Tirupathi, Andhra Pradesh, India 517502

E-mail: ¹kalyani.mft@gmail.com

Abstract

Mushrooms have been recognised universally as a highly nutritive food and are gathering more importance as functional food. Button mushrooms are the only natural vitamin-D source for vegetarians. Mushrooms were endowed with plentiful nutritional and functional benefits. Radiation is one of the novel food processing and promising technology. Food processing by the application of irradiation was non-thermal food preservation with minimal modifications regarding quality and sensory attributes. The irradiation of mushrooms can be a safe and cost effective method to enhance shelf life as well as to ensure hygienic and sensory quality. In the current study low dose of gamma irradiation i.e., 0.25 and 0.75 kGy was employed for mushrooms. Vitamin-D content was estimated in irradiated mushrooms at 0.75 kGy, was 3.92 µg, at 0.25 kGy was, 1.44µg and 2.77µg non-irradiated mushrooms. The other bioactive components i.e., antioxidant activity of mushrooms was enhanced during storage and better retention of vitamin-C and folic acid was noticed in irradiated mushrooms than non-irradiated mushrooms. The bioavailability of vitamin-D can enriched by the gamma irradiation process, it helps as a good source of vitamin-D and also it extended the shelf life of the mushroom. The application of gamma irradiation enhances the vitamin-D in mushroom by converting the endogenous ergosterol to ergocalciferol (Vitamin D₂). An irradiated mushroom was safe as well as with some added additional advantages.

Keywords: Processing, Radiation, Vitamin-D, Shelf life, Enhance.

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1. INTRODUCTION

Button mushroom (*Agaricus bisporus*) is the most popular variety, fetches high price, still dominating the Indian and International market. It contributes about 90% of total countries production and its global share is about 40%. Furthermore, macro fungi have a history of traditional use in oriental therapies and modern clinical practices continue to rely on mushroom-derived preparations. Mushrooms are potential sources of protein, essential amino acids, minerals and fiber are really valuable, excellent sources of most B-vitamins, key natural sources of ergosterol or pro-vitamin-D, antioxidant and anticancer components, they are used as food, food additives and as supplements. They accumulate a variety of bioactive metabolites e.g. phenolic compounds, polyketides, terpenes, steroids and polysaccharides. Presently, mushroom has been

recognized universally as a highly nutritive food and is getting more importance as medicinal/functional food.

Mushrooms are the only non-animal-based food containing vitamin D in the form of pro-vitamin D₂ (ergosterol), hence natural vitamin D sources for vegetarians was mushrooms. Vitamin D consists of two different compounds, vitamin D₂ from ergosterol and vitamin D₃ from 7-dehydrocholesterol. Vitamin D present in mushrooms converts the endogenous ergosterol to ergocalciferol (vitamin D₂) enhances through the use of artificial or exposure to UV light can increase vitamin D₂ content in mushrooms.

Ergosterol → Pre-vitamin D₂ → Vitamin D₂ (ergocalciferol)

Vitamin D₂ is the form of vitamin D that could be provided from mushrooms, and this form has some remarkable advantages over vitamin D₃. Vitamin D₂ is more effective for bone

mineralization than vitamin D₃, and vitamin D₂ is less toxic compared with vitamin D₃ (Jasinghe and Perera, 2005). Vitamin D is one of the important vitamins; it plays a key role in muscle function and immune system. The active form of Vitamin D is known as vitamin D₃, or 1,25-dihydroxycholecalciferol. This is derived either from diet or ultraviolet B (UVB) light conversion of 7-dehydrocholesterol in the skin, which is then hydroxylated to 25-hydroxyvitamin D in the liver, and then further hydroxylated to 1,25-dihydroxycholecalciferol in the kidneys. The main dietary source of vitamin D has long been thought to be cod liver oil and oily fish. Edible mushrooms have been shown to be abundant in ergocalciferol, which can then be hydroxylated to 25-hydroxyvitamin D in the liver. This can be further hydroxylated to the active form, 1,25dihydroxyvitamin D, in the kidneys. It is known that ingestion of edible mushrooms can result in an increase in serum vitamin D levels in humans, and it is also known that irradiating mushrooms with UVB light leads to an increase in their vitamin D content. Furthermore, in vivo studies in rats have shown an increase in both serum 25-hydroxyvitamin D levels and bone mineral density following ingestion of UVB-irradiated mushrooms.

Mushrooms are internationally regarded as poor man's meat because they are good substitute for meat which peasants cannot afford. The demand for mushrooms is growing day by day. But due to their perishable nature they spoiled very quickly. The maximum shelf life of mushrooms is 2-6 days at refrigeration (4⁰c) temperature (Vanitha et al., 2013). Their shelf-life is short due to postharvest changes, such as browning, cap opening, strip elongation, cap diameter increase, weight loss and texture changes, to their high respiration rate and lack of physical protection to avoid water loss or microbial attack. Thus prolonging postharvest storage, while preserving their quality, would benefit the mushroom industry as well as consumers. Based on the mushrooms nutritional importance and medicinal value, there is a need to increase the shelf life of

mushrooms by using advanced technology without affecting the functional components (Beaulieu et al., 1992).

There has been extensive research on finding the most appropriate technology for mushrooms preservation. Chemical treatments, refrigeration, coating, modified/controlled atmosphere packaging, use of humectants, use of tyrosine inhibitors and ozone treatment are frequently applied methods. Moreover, the majority of food preservation techniques operate by slowing down or inhibiting the growth of microorganisms. In contrast, Gamma, electron-beam and UV irradiation have been shown to be potential tools in extending the postharvest shelf-life of fresh mushrooms, inhibit or inactivate microbial growth completely, resulting in commercially sterile and shelf-stable food.

Food irradiation technology has unique merits over conventional methods of preservation as this process does not lead to loss of flavor, odor, texture, and freshness. Unlike chemical fumigants, irradiation does not leave any harmful toxic residues in food and is more effective. It is efficient and can be used to treat pre-packed commodities. Export development authorities, commodity boards, food industry, farmers, traders, and exporters of agricultural commodities can be benefited from the use of radiation processing technology.

Now a day's people are showing more interest on fresh fruits and vegetable consumption due to the presence of functional components which helps in treating/reducing the risk of diseases, and they act as functional foods. By irradiation processes the valuable functional components can be retained in natural form and also extend the shelf life of mushrooms. Gamma-irradiation alone or in combination with refrigeration has been shown to prolong shelf-life through reducing moisture loss and improving color and appearance (Ajilouni et al., 1993).

As Food irradiation is one of the best and safest food preservation techniques designed to ensure the provision of better quality with an extended shelf life. Keeping in view of the

above points, the present study carried out to study the effect of radiation processing on functional components and shelf life of Mushrooms.

2. MATERIALS AND METHODS

Sample collection:

Freshly harvested, mature mushrooms (*Agaricus bisporus*) of similar size and free from physical defects were obtained from commercial mushroom growers. Immediately after harvesting, mushrooms were cleaned and then packed in high density polyethylene covers each with 200g due to light weight and also to avoid the damage of mushrooms during storage and processing.

Irradiation of mushrooms:

The irradiation was done in gamma irradiation plant at food irradiation unit at Quality control lab, Acharya N.G. Ranga Agricultural University; Hyderabad. The equipment used for radiation processing was Gamma (δ) Irradiation chamber, Model- GC-5000. The source for the radiation processing was cobalt - 60. In the present work low dose levels were used for radiation processing i.e., 0.25 kGy and 0.75 kGy.

Functional Components: The functional parameters were analyzed for non-irradiated and irradiated mushrooms. The predominant functional components such as vitamin-C, folic acid, vitamin-D and total antioxidants activity of mushrooms were analyzed. Vitamin-C and folic acid was estimated by procedure followed by Ranganna (2001). Total Antioxidant Activity in mushrooms was estimated by using TBARS method. Vitamin-D content in the mushrooms was estimated by using Ultra-High-Performance Liquid Chromatography/Tandem Mass Spectrometry First Action 2011, AOAC method No.2011.11 (LC-MS/MS).

Experimental Period: The irradiated mushrooms at 0.25 kGy (**I₁**), 0.75 kGy (**I₂**) and non-irradiated mushrooms (**NI**) was stored for a period of 21 days. The analysis was done at

initially (**Initial phase**) and at the end of the experimental period (**Final phase**). The results of the experiment was compared between the initial and final phase of the study and discussed in results and discussion.

3. RESULTS AND DISCUSSION

Proximate components did not alter or typically not much dissimilarity was observed during processing, by this reason this study was majorly focused on functional components. Vitamin-C, folic acid, vitamin-D and total antioxidants activity of Mushrooms were analysed in the present study and the results were discussed in following heads.

Vitamin-C

Vitamin-C (mg/100g) in vegetables has varietal and functional factors. The vitamin-C of Mushrooms was analysed in non-irradiated and irradiated samples during initial and final phase of the experimental period. A sharp decrease in vitamin-C content was observed in non-irradiated Mushrooms (6.42mg to 5.67mg) and irradiated mushrooms at 0.25 (5.20mg to 4.87mg) and 0.75 kGy (4.58mg to 4.00mg) from initial to final phase of the experimental period (Fig.1).

The difference in vitamin-C content between and among irradiated samples (0.25 and 0.75 kGy) and non-irradiated samples was significant ($p < 0.05$) from initial to final phase of the experimental period.

Vitamin-C was in the form of ascorbic acid. The reason for accelerated decrease of Vitamin-C in irradiated and non-irradiated samples might be enhanced respiration result in increased enzymatic activity causing rapid degradation of ascorbic acid. The changes in reduced ascorbic acid are due to its role as a radical scavenger. Thayer and Rajjkowski (1999), revealed that irradiation oxidized a portion of total ascorbic acid to dehydro form and both of these forms of vitamins are biologically active, suggesting minimal nutritional impact.

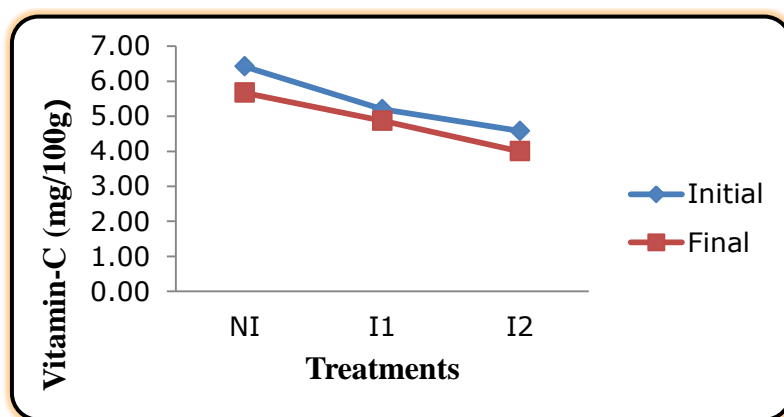


Figure 1 – Effect of Gamma Irradiation on Vitamin-C content of Mushrooms

Similar trend was observed by Hajara *et al.*, (2006), in their study on radiation processing of minimally processed carrot and cucumber. The minimally processed carrot and cucumber at a dose of 2 kGy was carried out over a storage period of 16 days at 8⁰C and 10⁰C. During storage both the control as well as the irradiated samples showed significant decrease in vitamin-C content.

The vitamin-C content decreased in all the samples on storage. Loss of vitamin-C was more in non-irradiated samples and even though the loss of vitamin-C was observed in irradiated Mushrooms it was comparatively less when compared to non-irradiated Mushrooms. The stability and variation of ascorbic acid not only depends on the irradiation treatment and doses, but also environmental factors such as storage, temperature etc.

Vitamin-C (ascorbic acid) exhibits a high

degree of sensitivity to ionizing radiation. Furthermore, it is known to be readily oxidized to dehydro ascorbic acid on irradiation (Stewart, 2001 and Song *et al.*, 2006).

Folic Acid

The folic acid ($\mu\text{g}/100\text{g}$) of Mushrooms was estimated in non- irradiated and irradiated samples. Folic acid content of Mushrooms was estimated at initial and final phase of the experimental period and the results. The decrease in folic acid content of Mushrooms was observed in all the treated and non-treated samples from initial to final phase of the experimental period (fig 2) and it was statistically significant ($p < 0.01$) in non-irradiated Mushrooms, whereas in irradiated Mushrooms it was not significant. At the end of the experimental period no significant difference was observed in folic acid content of Mushrooms irrespective of the treatments.

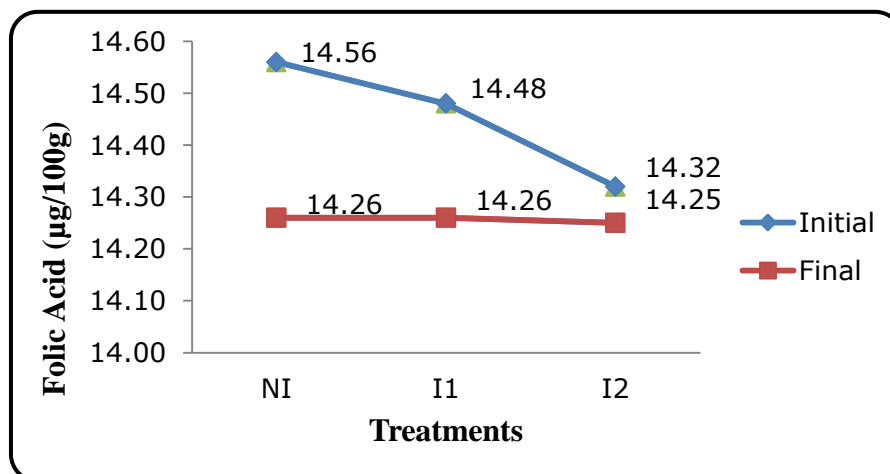


Figure 2 – Effect of Gamma Irradiation on Folic acid content of Mushrooms

These findings are in support with the results of Lester *et al.*, (2010), in his study on the effect of gamma-irradiation on ascorbic acid, carotenoids, folate, α -tocopherol and phyloquinone concentrations of baby-leaf spinach. There is no evidence in the literature that transient sunlight exposure increases folate levels in Mushrooms or other plants. However, all folates are to various degrees unstable and are particularly sensitive to oxidation. Despite the fact that folates are good absorbers of UVB and UVA light, these compounds are believed to be relatively stable to UV light exposure, but degradation of 5-methyltetrahydrofolate in the presence of UV light has been reported in the presence of photo sensitizers such as riboflavin (Simon *et al.*, 2011).

In the present study, folic acid content of Mushrooms is not affected by irradiation processing. Initially slight decrease in irradiated Mushrooms was noticed and at final phase the maximum retention of folic acid was observed in irradiated Mushrooms.

Vitamin-D

Mushrooms are the only vegetative source of vitamin-D. Immediately after irradiation, significant difference was showed between non-irradiated and irradiated samples of Mushrooms. The Vitamin-D analysis was done at initial and final phase of the experimental period as indicated for both irradiated and non-irradiated Mushroom. The decrease of vitamin-

D was more in non-irradiated (2.77 μ g to 1.97 μ g) when compared to irradiated Mushrooms at 0.25(1.44 μ g to 1.28 μ g) and 0.75 kGy (3.92 μ g to 3.02 μ g) from initial to final phase of the experimental period. Statistically a significant difference was observed in vitamin-D content of both non-irradiated and irradiated Mushrooms during the experimental period from initial to final phase. At the end of experimental period significant difference ($p < 0.01$) was observed in vitamin-D content of Mushrooms between the treatments.

The increasing trend of vitamin-D was observed with the increase of dosage in Mushrooms. The increase of vitamin-D was more in Mushrooms irradiated at 0.75 kGy followed by 0.25 kGy and non-irradiated Mushrooms (Fig. 3).

The temperature and exposure time of irradiation plays an important role in the conversion and this may be one of the reasons. Irradiation also contributes to an oxidative atmosphere and prolonged exposure of vitamin-D to UV radiation may result in photo-degradation at vitamin-D₂. Another important factor is moisture, at low moisture levels, the specific surface area of the tissue is increased, results in the oxidation of vitamin-D₂. Furthermore, irradiation also contributes to oxidative atmosphere, and photo-degradation of vitamin-D₂ may occur.

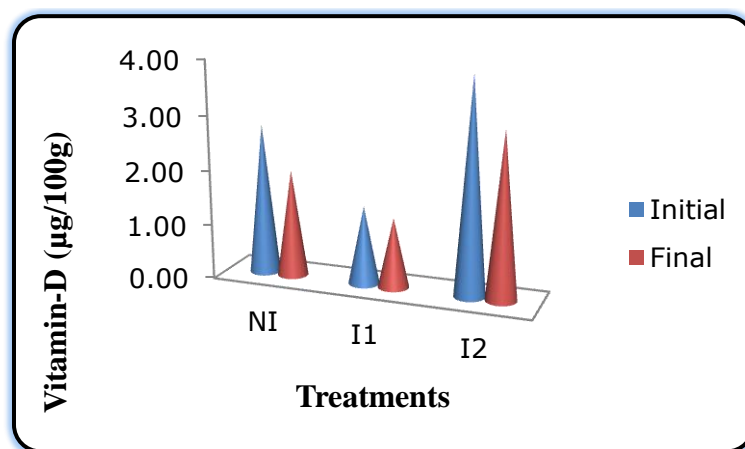


Figure 3 – Effect of Gamma Irradiation on Vitamin-D content of Mushrooms

These findings are in support with the results of Jasinghe and Perera (2005). They conducted a study on Distribution of ergosterol in different tissues of Mushrooms and its effect on the conversion of ergosterol to vitamin-D₂ by UV irradiation. In this study, highest ergosterol content was found in button Mushrooms (7.80 mg/g DM) while the lowest was in enoki Mushrooms (0.68 mg/g DM). The conversion of ergosterol to vitamin D₂ was about four times higher when gills were exposed to UV-A irradiation than when the outer caps were exposed to the same.

Kalaras *et al.*, (2011), carried out a study on Effects of Postharvest Pulsed UV Light treatment of White Button Mushrooms (*Agaricus bisporus*) on Vitamin D₂ Content and Quality Attributes. In this study, dose-response suggests a non-linear relationship between PUV irradiation dose and vitamin-D₂ content of fresh Mushrooms. The initial levels of vitamin D₂ in untreated Mushrooms were less than 0.005 µg/g DW, and rapidly increased to 12.6 µg/g DW after 3 pulses. The D₂ produced with 3 pulses decreased after 3 days of storage.

Souci *et al.*, (1989), reported that ergosterol contents and the conversion rate of ergosterol to vitamin D₂ in different types of Mushrooms were varied. Button Mushrooms have lower vitamin D₂ content compared to other types of edible Mushrooms. This may be due to the gill was not exposed. The gill of sliced button Mushroom was exposed to UV-B; therefore, Vitamin-D₂ concentration of sliced button

Mushrooms was higher than that of whole Mushroom.

Irradiation shows significant effect on vitamin-D content in Mushrooms. The increase of vitamin-D was more in irradiated Mushrooms at 0.75 kGy than other samples. The conversion of vitamin-D was more in 0.75 kGy irradiated Mushroom. A slight decrease was observed during the experimental period. The conversion was affected by many factors. Exposure time, temperature, dosage of Irradiation and type or mode of raw material significantly affected the vitamin-D content in Mushrooms.

Total Antioxidant activity

The antioxidants are recognized as bio-active compounds that act against possible ill effects of free-radical damages in humans. The antioxidant activity of a compound has been attributed to various mechanisms.

At initial and final phase of the experimental period total antioxidant activity was estimated in Mushrooms (Fig.4). The high increasing trend of total antioxidant activity was observed in irradiated Mushrooms at 0.25 kGy (35.80% to 52.02%), whereas a very minute increase was there in irradiated Mushrooms at 0.75 kGy (41.73% to 52.05%) when compared with the non-irradiated (43.02% to 45.60%) from initial to final phase of experiment. The significant difference was observed among all the samples of Mushrooms during the experimental period at initial and final phases.

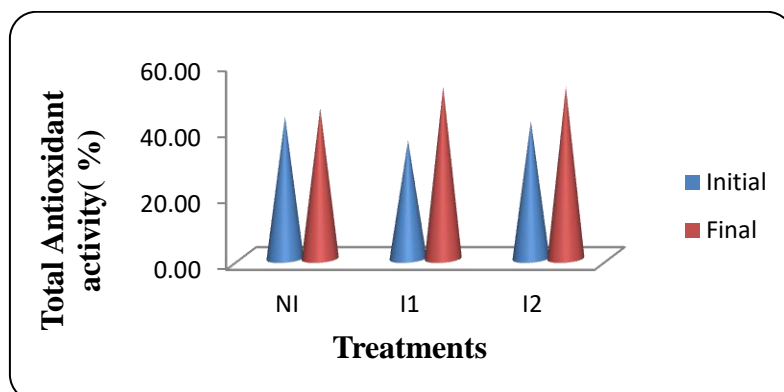


Figure 4 – Effect of Gamma Irradiation on Total Antioxidant activity of Mushrooms

Irradiation increased the antioxidant activity during the experimental period (fig 4), it is also possible that the increased antioxidant capacity is related to tissue browning. Another reason for improved antioxidant activity could be due to formation of novel compounds having antioxidant activity during processing. Non-enzymatic browning reaction products might be formed during prolonged exposure with the improvement of antioxidant activity in Mushrooms.

Another reason for the increase of total antioxidant activity might be due to the fact that the irradiation disrupts the cell wall and liberates antioxidant compounds from insoluble portions of Mushroom, which in turn, increase the pool of bio accessible antioxidant compounds.

Song *et al.*, (2006), reported that the total phenols analyzed in irradiated Kale Juice immediately after the irradiation, was significantly lower than the control. However, the phenolic compound level of the irradiated sample becomes higher after one day than that of the control. The phenomenon was attributed to the immediate oxidation of the phenolic compounds, thus playing an antioxidant role by reducing the free radicals and the reactive oxygen species induced by irradiation.

Adamo *et al.*, (2004), have opined that the destructive processes of oxidation and γ -irradiation are capable of breaking the chemical bonds of polyphenols, thereby releasing soluble phenols of low molecular weights, leading to an increase of antioxidant-rich phenolics. The decrease of antioxidants is attributed to the formation of radiation-induced degradation products or the formation of free radicals.

Mami *et al.*, (2013), carried out a study on improvement of shelf-life and postharvest quality of white button Mushroom by ^{60}Co γ -ray irradiation. Five different doses of gamma irradiation, including: 0 as control, 0.5, 1, 1.5 and 2 kGy were used. Irradiation increased both phenolics content and antioxidant capacity, suggesting the increased phenolics synthesis contributed to the total antioxidant capacity. It is also possible that the increased

antioxidant capacity is related to tissue browning. It is well known that irradiation inactivates food borne pathogens in various vegetables, resulting in improved microbial food safety of fresh-cut vegetables.

In the current study irradiation has significant effect on total antioxidant activity of Mushrooms. The decrease of total antioxidant activity was observed initially in irradiated Mushrooms which might be due to immediate oxidation of the phenolic compounds. On storage the total antioxidant activity was increased more in irradiated Mushrooms than non-irradiated Mushrooms. A reason for improved antioxidant activity could be due to pool of bio accessible antioxidant compounds.

4. CONCLUSION

The current study reveals that the effect of gamma irradiation at low doses of functional components of mushrooms was analyzed at initial and final phases of experiment. Mushrooms irradiated at 0.75 kGy was more optimum in improving the vitamin-D and total antioxidant activity, no vast discrepancy or loss was noticed in vitamin-C and folic acid content of mushrooms when compared with initial to final phase of experiment. They were least affected by irradiation and retention of nutrients was observed during storage period. It was quite evident that gamma irradiation improves the shelf life of the vegetables as well as improves their quality in terms of physical, nutritional, functional and safety aspects.

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