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Improvement of shelf-life and postharvest quality of white button mushroom by ^{60}Co γ-ray irradiation

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Abstract

This project was done to study the effect of irradiation on shelf-life and post-harvest quality of *A. bisporus*. Five different doses of gamma irradiation, including: 0 as control, 0.5, 1, 1.5 and 2 kGy were used. The experiment was conducted using a ^{60}Co gamma-ray source facility, PX-30 at a dose rate of 0.22 Gy/sec and measurements were made during 1, 4th, 8th, 12th and 16th day for the mushrooms stored continuously at 4 °C and 80% relative humidity. There were significant differences between irradiated and non-irradiated (control) mushrooms in evaluated indices. Analyses of phenolic compounds revealed that mushrooms in doses of 1.5 and 1 kGy contained more phenols than 0, 0.5 and 2. The lowest amount of antioxidant capacity was observed in non-irradiated mushrooms. In addition, treatment of 1.5 kGy caused the highest TSS. There were significant differences in mushroom L* value in storage times of 12 and 16 among different doses. Irrigated mushrooms with 1.5 and 1 kGy appeared the more L*value compared with other treatments. Also, amounts of protein content, electrolyte leakage rate, weight loss and Vitamin C were significant in different storage times. Increased shelf-life of *A. bisporus* can be achieved by application of suitable doses of ^{60}Co γ-ray irradiation. Results suggest that irradiation also increased nutritional quality of button mushroom. The data increase the current understanding of the effects of γ-ray irradiation on the biochemical changes associated with postharvest senescence and should lead to more targeted strategies for reducing postharvest quality loss in *Agaricus bisporus.*

Keywords: *Agaricus bisporus*, γ-ray, dose, quality and shelf-life.
Abbreviations: L*_Lightness.

Introduction

The button mushroom [*Agaricus bisporus* (Lange) Sing)] is the most widely cultivated and consumed mushroom throughout the world and includes about 40% of total world mushroom production (Giri and Prasad, 2007). The production and fresh use of button mushroom in Iran have increased rapidly during the last decade. However, the high perishable nature of mushrooms remains a problem for the progress of this industry (Beaulieu et al., 1992; Gautam et al., 1998). In fact, fresh mushrooms can only be stored for a few days until they lose freshness and quality. There are many methods to extend the shelf-life of mushrooms. They include modified atmosphere packaging (MAP) (Roy et al., 1995), controlled atmosphere storage (CA) (Lopez-Briones et al., 1992), coating (Nussinovitch and Kampf, 1993), refrigeration (Gormley, 1975; Mau et al., 1993), cultivating with CaCl2 solution (Miklus and Beelman, 1996) and using sorbitol (Roy et al., 1995). Although CA storage is effective to lower respiration rate and increases shelf-life of fruits and vegetables, it is not appropriate for vegetables, as mushrooms, which have extremely high respiration rates (Roy et al., 1995). Also, authors found that MAP could have a damaging effect, causing anaerobic respiration as well as potential growth of anaerobic pathogens (Beit-Halachmy and Mannheim, 1992; Varoquaux et al., 1999). A potentially attractive alternative is exposure to ionizing radiation, and previous papers have suggested this method is highly effective in inhibiting physical changes associated with postharvest deterioration and maintaining a fresh product appearance (Kader, 1986). Food processing by employing radiation is well established as a physical, non-thermal mode of food preservation (cold-pasteurization) that processes foods at or nearly at ambient temperature. Irradiation of food products causes minimal modification in the flavor, color, nutrients, taste, and other quality attributes of food. However, the levels of modification (in flavor, color nutrients, taste etc.) might vary depending on the basic raw material used, irradiation dose delivered, and on the type of radiation source employed (gamma, X-ray, UV, electron beam) (Bhat and Sridhar, 2008; Bhat et al., 2007; Mexis et al., 2009). Gammas are short wave length, high energy photons, and have deep penetrating power. Gamma rays come from spontaneous disintegration of radioactive nuclides (Cobalt 60 or Cesium 137) as their energy source. During irradiation, the radioactive nuclides are pulled out of storage (water pool) into a chamber with concrete walls that keep any gamma rays from escaping (Park, 2002). International agencies including IAEA, FAO and WHO concluded that irradiation of any food commodity up to a dose of 10 kGy exhibits no health risks (WHO report, 1981; Diehl, 2002). Gamma-irradiation alone and in combination with refrigeration has been shown to prolong shelf life through reducing moisture loss and improving color and appearance (Ajlouni et al., 1993). Doses

of γ-irradiation inhibited cap opening, stalk elongation and browning and reduced the level of microbial contamination of *Agaricus bisporus* (Gill et al., 1969). Electron-beam irradiation levels above 0.5 kGy reduced total plate counts, yeast and mold, and psychrotrophic counts to below detectable levels and prevented microbial- induced browning. Although, color was preserved by irradiation as evidenced by the higher *L** values, they showed that the irradiation at 1 kGy was most effective in extending shelf-life of mushroom slices (Koorapati et al. 2004)**.** Xiong et al. (2009) indicated that a ^{60}Co g-irradiation dose of 1.2 kGy significantly delayed (by 6–9 days) the onset of fruit body softening, splitting and browning compared with non-irradiated controls and test samples subjected to lower or higher irradiation doses. Irradiation with 1.2 and 1.6 kGy also had a positive effect on other indicators of mushroom tissue senescence, resulting in smaller decreases in soluble protein levels and more protracted increases in proteinase activity. Furthermore, g-irradiation extended the storage life of Pleurotus sajor-caju (Roy et al., 2000) and Pleurotus pulmonarius (Xia et al., 2005) without adversely affecting key nutritional components. Radiation treatments also delayed cap opening and browning, and lowered the rates of decomposition and weight loss, in the straw mushroom, Volvariella volvacea (Liu et al., 2003; Ye et al., 2000). Fan (2005) found that irradiation increased the phenolic content and antioxidant capacity of both tissue types of all vegetables at day 4 and day 8. His results suggest that irradiation increased nutritional quality of leafy vegetables, but some adverse visual quality changes were encountered. In addition, it was concluded that irradiation treatments of carrot and kale juice improve the microbiological safety with maintaining or even enhancing the antioxidative activity (Song et al. 2006). The aim of this investigation was mainly directed to the effect of different doses of gamma-irradiation on the shelf-life and postharvest quality of button mushroom.

Results and Discussion

Effect of gamma irradiation on mushroom whiteness

Our results indicated that different doses of gamma irradiation affected the shelf-life and postharvest quality (Tables 1 and 2). During the storage, decreases of the mushroom whiteness were observed for all mushrooms, control and treated. In the times of 12 and 15, color change was significant ($P \leq 0.01$). The *L** values in C (0 kGy) decreased from 87 on day 1 to 66 on day 16. They decreased from 86 to 76, 87 to 79, 87 to 79 and 86 to 72 in 0.5, 1, 1.5and 2 kGy from day 1 to day 16, respectively (Fig 1). Whiteness of pileus and stipe is often used as important index of visible quality, since rapid discoloration occurs after harvest (Gormley, 1975). Most of the researchers agree that irradiated mushrooms retain their original skin color for longer periods or darken less rapidly than unirradiated mushrooms (Kova´cs and Vas, 1974; Thomas, 1988). The coloration change in mushrooms upon irradiation is still the subject of some controversies.

Effect of gamma irradiation on weight loss

Weight loss is one of the physiological parameters used as quality indicator in fruits. Weight losses in controls and irradiated samples increased in parallel during the experimental storage period. Final weight losses recorded in control samples (0 kGy) were not significantly different from those observed in irradiated mushrooms, except 2 kGy (fig 2). The weight loss is because of evaporation of water from the fruit surface as a result of respiration and transpiration.

Effect of gamma irradiation on electrolyte leakage rate

Electrolyte leakage rate of mushrooms were lowered as significantly by application of 0.5, 1 and 1.5 compared with control in storage times, except days of 1 and 12 that 0.5 kGy had not significant difference with control. The treatment of 2 kGy caused a negative effect on electrolyte leakage rate unlike other gamma doses (fig 3). Electrolyte leakage is an index of the semipermeable properties of cell membranes, and a reduction in membrane integrity resulting from lipid peroxidation increases membrane leakage and enhances cell senescence (Hildebrand, 1989). Decreased rates of membrane lipid peroxidation and membrane leakage observed following treatment of *V.* V*olV*a*cea* and *L. edodes* fruit bodies with 60Co irradiation (Ye et al., 2000) and calcium chloride (Li et al., 2000)), respectively, were accompanied by marked prolongation of postharvest mushroom freshness. Fan and Sokorai (2002) found that irradiation increased electrolyte leakage in fresh-cut Iceberg lettuce. Voisine et al. (1993) showed 2 kGy gamma radiation increased electrolyte leakage in cauliflower.

Effect of gamma irradiation on total soluble solid

Our investigations suggest a significant decrease in the total soluble solid with 1, 1.5 and 2 kGy as well as control. Dose of 0.5 more kept the amount of TSS in storage time. The mushrooms which treated with 2 kGy had the lower TSS compared with other treatments (fig 4). Total soluble solid was earlier reported to be the major respiration substrate in *A. bisporus* during postharvest storage (Hammond and Nicols 1975), and steady decreases in the soluble solids concentration were previously reported in fruit bodies stored at cold-temperatures (Tseng and Mau, 1999). Radiation effects on TSS in mushrooms have not been reported yet.

Effect of gamma irradiation on vitamin C and phenol content

Researchers showed that irradiation increases phenolic content in vegetables (Qufedjikh *et al.,* 2000; Fan and Sokorai, 2002), which may in turn influence appearance, flavor and nutritive values. In this study, irradiation also significantly enhanced total phenols content, especially between days 1 and 4, whereas Vit. C was lowered (figs 5 and 6). Several authors noted a decrease in vitamin C in both stored irradiated or unirradiated vegetable crops (Rai and Saxena, 1988., Salem, 1974). Gamma radiation causes a notable damage on vitamin C of *A. bisporus* but the changes which occurred during eighth day of storage, irradiated mushrooms were similar to those of the non-irradiated at temperature 4°C. It is the most difficult vitamin to preserve during storage. The possible reason for accelerated decrease of ascorbic acid in irradiated and no irradiated samples might be enhanced respiration resulting in increased enzymatic activity causing rapid degradation of ascorbic acid; irradiation also could not control the loss of ascorbic acid. Phenolic compounds are generally synthesized by the shikimate pathway in which PAL is the key enzyme.Similar to mechanical wounding (Kang and Saltveit, 2002), irradiation can increase PAL activity of plant tissue (Tomas-Barberan and Espin, 2001), resulting in the accumulation of phenolic compounds. The ability of gamma irradiation to increase polyphenolic acids in plant metabolites has also been

Table 1. ANOVA effects due to irradiation with five different doses (0, 0.5, 1, 1.5 and 2 kGy) on total soluble solid (TSS), vitamin C content, protein content, antioxidant capacity (AC) and total phenolic compounds.

Source	df	TSS (g/100g fwt)	Vit C. (mg/100g fwt)	Protein (g/100g fwt)	AC (mmol/kg fwt)	Total phenol (mg/ 100g fwt)
A	4	1.156**	0.251**	0.126**	0.161**	9248.746**
Error (a)	10	0.009	0.003	0.0	0.012	78.12
B	4	3.659	1.311	1.577	0.756	14092.113
a*b	16	0.073**	0.024**	0.014**	0.017**	388.98**
B	8	0.031	0.001	0.0	0.002	42.043
C.V	-	2.39	3.84	1.956	2.56	3.39

ns, ** not significant or significant at P≤0.01, ANOVA.

observed in soybeans samples treated with γ-irradiation at levels ranging from 50 to 150 Gy increased free polyphenolic acids (Variyar *et al.,* 2004). Variyar *et al.* (2004) also suggested a radiation induced breakdown of glycosides resulting in the release of free isoflavones. Isoflavones are phenolic compounds, and they were increased in their concentration at a low dose of γ-irradiation supported by the corresponding increase in the total phenolic content. Siddhuraju *et al.* (2002) attributed such increase in polyphenolic acids to higher extractability by depolymerization and dissolution of cell wall polysaccharides due to gamma irradiation. Fan *et al.* (2003) reported that the free radicals generated in plants during irradiation may act as stress signals and may trigger stress responses in plants, resulting to increased polyphenolic acid synthesis which had notable antioxidative properties.

Effect of gamma irradiation on protein

The amounts of protein continually decreased from day 1 to day 16. The highest protein in storage times of 4, 8, 12 and 16 in mushrooms was due to the use of 1.5 kGy. In first day of storage the dose of 0.5 had only significant difference with other treatments (fig 7). Murr and Morris 1975, had pointed that protein degradation, as indicated by protease activity and the level of free amino acid in the tissue, increased during postharvest maturation of the mushroom, and they assumed that the assimilation may function as the source of C or N. A decline in soluble protein concentration is considered to be an important indicator of tissue senescence (Burton et al., 1997). The small increases in proteinase activity occur in samples treated with irradiation (Xing et al. 2007); therefore gamma irradiation improved protein after day 1 compared with controls. In doses of higher than 1.5 decreases may be induced by protein denaturation (Arntfield and murray, 1981).

Effect of gamma irradiation on antioxidant capacity

The lowest amount of antioxidant capacity was observed in control. AC did not differ significantly with 0 and 0.5 kGy, except day12 of storage (fig 8). Major antioxidants in fresh vegetable are phenolic acids and flavonoids (Hanasaki et al., 1994). Phenolics, such as tannic acid and gallic acid, have high antioxidant activity (Pulido et al., 2000). 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 (Thayer and Rajkowski, 1999).

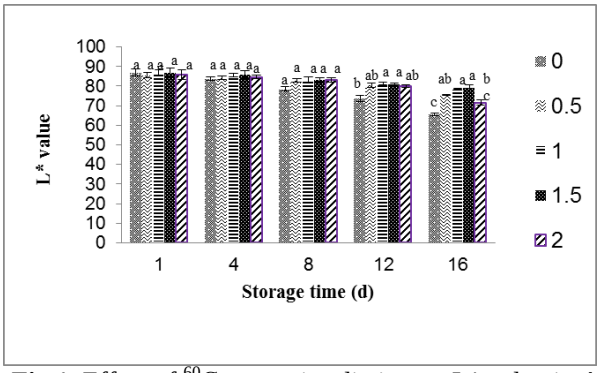

Fig 1. Effect of ^{60}Co γ-ray irradiation on L* value in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

Materials and methods

Study site

The experiments were conducted in the Agricultural Faculty of Guilan University; Rasht and Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran, in 2011-2012. The experiment was set up in a Split Plot in Time (SP-T) design with 3 replications.

Mushroom samples

Freshly harvested, mature sporophores of *Agaricus bisporus* of similar size and free from physical defects were obtained from a commercial mushroom-growing operation (Bibi) located near Karaj. Immediately after harvesting, fruit bodies were packed into polystyrene trays (20_10 _1 cm), covered with plastic film, stored at 4 °C and transported to the irradiation center of the Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, IRAN. The period between harvesting and irradiation was approximately 4 h. Each replication was containing 10 trays with 200g in weight.

Irradiation doses

Fruit bodies (200 g) were placed in plastic trays and irradiated at 20 °C. Irradiation was carried out using a ^{60}Co gamma-ray source facility, PX-30, made in Russia at a dose rate of 0.22 Gy/sec. The correction for the decay of ^{60}Co radionuclide and the subsequent decrease of the dose rate was performed. The samples were irradiated under various dose intervals of 0.5, 1, 1.5 and 2.0 kGy. The dosimetry was made using the Fricke reference standard dosimetry system (Holm and Berry, 1970).

Table 2. ANOVA effects due to irradiation with five different doses (0, 0.5, 1, 1.5 and 2 kGy) on color (*L*, *a*, *b* values), weight loss (WL) and electrolyte leakage rate (EL).

Source	df	WL (%)	EL (%)	*L** values	a* values	b* values
A	4	0.413**	1.415^{**}	75.468^{**}	10.903^{ns}	9^{ns}
Error (a)	10	0.024	0.009	13.283	3.393	20.414
B	4	138.305	2.013	356.166	10.919	155.506
a*b	16	0.063**	0.106**	16.967**	2.174^{ns}	4.926^{ns}
B	8	0.007	0.006	6.935	0.578	2.614
C.V	-	1.53	1.9	1.65	38.42	5.22

ns, ** not significant or significant at P≤0.01, ANOVA.

Measurements were made during 1, 4th, 8th, 12th and 16th day for the mushrooms stored continuously at 4 °C. Ten mushrooms were measured from each treatment-day interaction.

Color measurements

Sample color was measured at specified time intervals during storage period by a model colorimeter (Minolta CR-400, Japan). In this system of color representation the values L*, a* and b* describe a uniform three-dimensional color space, where the L* value corresponds to a dark-bright scale, a* is negative for green and positive for red, whereas b* is negative for blue and positive for yellow. The colorimeter was calibrated using a standard white plate under normal light conditions.

Weight loss

Weight loss during postharvest storage was determined by periodical weighing, and calculated by dividing the weight change during storage by the original weight.

Weight loss (%) = $[(Wi\text{-}Ws)/Wi] \times 100$

Where Wi = initial weight and Ws=weight at sampling period.

Electrolyte Leakage Rate

Electrolyte leakage rate was measured essentially as described by Autio and Bramlage 1986. *H. marmoreus* fruit bodies (5 g) were cut into four pieces, leaving the pileus intact and suspended in 40 mL of deionized water in a 100 mL beaker. Electrical conductivity was measured immediately (*P*0) and again after 10 min (*P*1). Samples were then boiled for 10 min and cooled to room temperature, and a final conductivity measurement (*P*2) was taken. The relative electrolyte leakage rate (RELT) was calculated according to the following equation: (*P*1 – *P*0)/ (*P*2 – *P*0) and expressed as a percentage.

Total Soluble Solid (TSS)

TSS is an index of soluble solids concentration. Homogenated ground sporophore tissue was filtered through the filter paper Whatman No. 1 by means of vacuum and the total soluble solid of the flow throughout the process was determined by a digital refractometer ATAGO PR-32α (ATAGO, USA Inc., Kirkland, WA). The results were expressed as g /100g FW.

Vitamin C Content

Vitamin C was determined by the 2, 6-dichlorophenolindophenol titration method, in which the dye

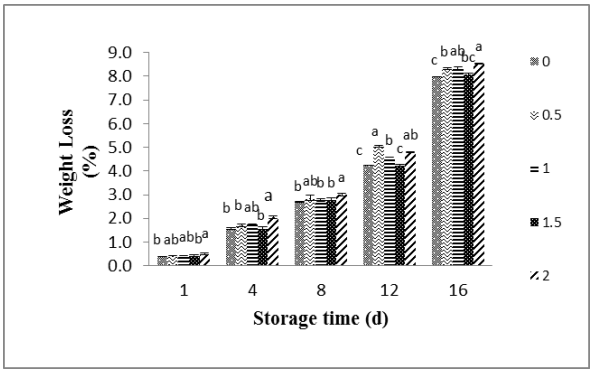

Fig 2. Effect of ^{60}Co γ-ray irradiation on weight loss in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

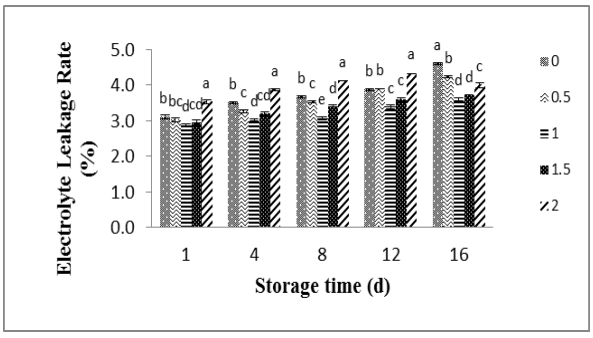

Fig 3. Effect of ^{60}Co γ-ray irradiation on electrolyte leakage rate in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

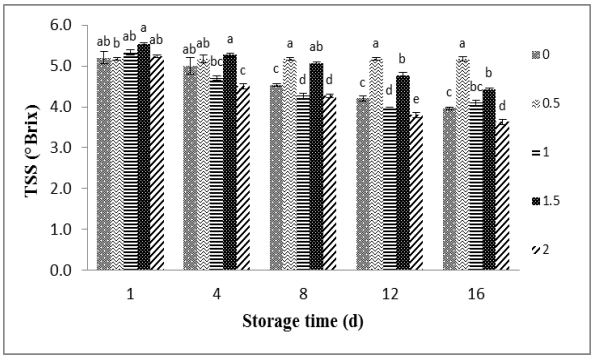

Fig 4. Effect of ^{60}Co γ-ray irradiation on total soluble solid (TSS) in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

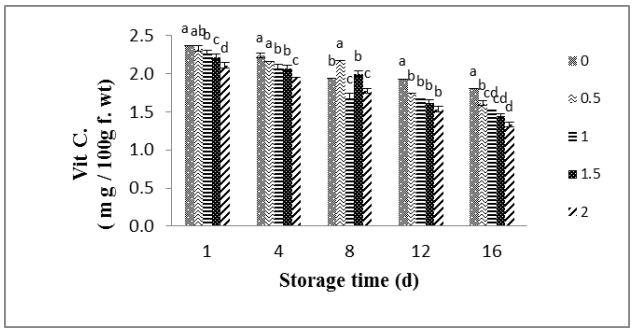

Fig 5. Effect of ^{60}Co γ-ray irradiation on Vit C in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

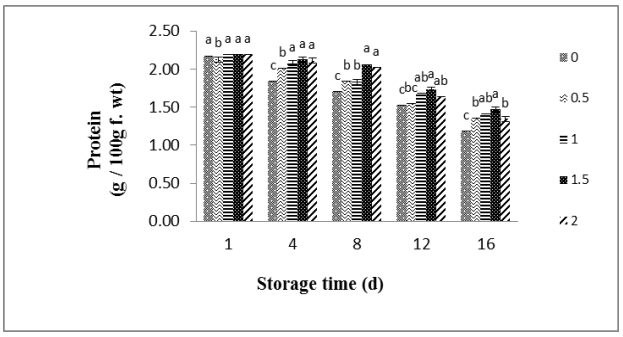

Fig7. Effect of ^{60}Co γ-ray irradiation on protein in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

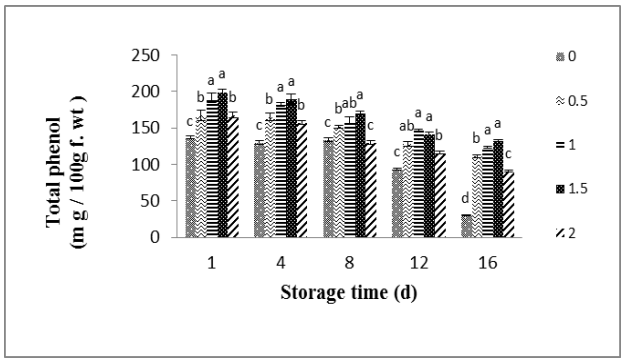

Fig 6. Effect of ^{60}Co γ-ray irradiation on total phenol in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

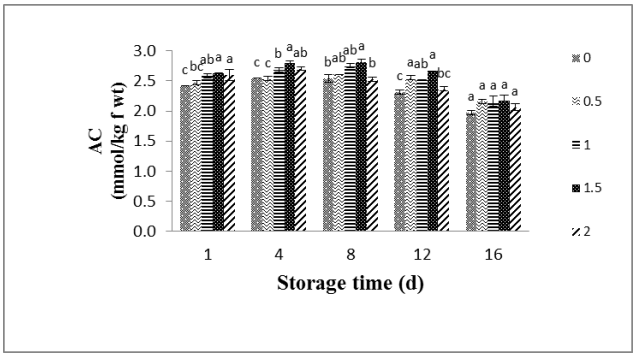

Fig 8. Effect of ^{60}Co γ-ray irradiation on antioxidant capacity (AC) in A. bisporus fruit bodies during storage at 4 °C. Vertical bars represent the standard deviation about the mean (r = 3).

is reduced by ascorbic acid, resulting in disappearance of the color (AOAC, 2002). Mushroom fresh tissue (3 g) was mixed with 20 mL (3%) metaphosphoric acid and thereafter was homogenized. Then ascorbic acid was determined by titration of 15 mL filtrated juice by DIP containing bicarbonate sodium an expressed by mg ascorbic acid /100g FW.

Protein Content

Protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard. The absorbance of blue colour was read at 595 nm using uv – visible spectrophotometer model PG Instrument +80, England. The amount of protein was quantified by using a standard curve and result were expressed as mg protein per 100g fresh weight of mushrooms.

Total Phenolic Compounds

Amounts of total phenolic compounds in fresh mushrooms were determined according to the Folin-Ciocalteu procedure (Singleton et al., 1990) with some modifications. For this purpose, 0.5 g mushroom slices was ground in the presence of liquid nitrogen by mortar and pestle, then 10 mL pure methanol were added to it for extracting phenolic compounds. The extract was centrifuged with 3000 rpm for 15 min at 4°C with an AVANTI™ J-25 centrifuge (Beckman Instruments Inc., Fullerton, CA) and then filtered through a Whatman no. 1 filter paper. Then 300 µl of methanolic extract was brought to a volume of 500 µl with distilled water into test tubes, followed by 2.5 mL of 10% Folin–Ciocalteu reagent and allowed to stand for 6 min. Thereafter, 2 mL of 7.5% sodium carbonate solution were added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm using an UV/Vis spectrophotometer model PG Instrument + 80, (Leicester, UK). Results are expressed as mg gallic acid/100 g fresh weight. Each assay was carried out in triplicate.

Antioxidant Capacity

The antioxidant capacity of fresh mushroom was analyzed on the base of determination of free radical-scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the procedure described by Elez-Martínez and Martín-Belloso (2007). Mushroom samples were centrifuged for 15 min at 4°C (Beckman Instruments Inc., Fullerton**,** California) and filtered through Whatman No. 1 filter paper. Aliquots of 0.01 mL of the supernatant was mixed with 3.9 mL of methanolic DPPH solution and 0.09 mL of distilled water. The homogenate was shaken vigorously and kept in the dark for 30 min. The absorption of the samples was measured with a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 515 nm for DPPH. The percentage of inhibition of the absorbance was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH value was calculated by using a calibration curve and the percentage of inhibition was determined.

Statistical Analysis

The data were subjected to Analysis of Variance in SAS (ver. 9. SAS, Inc., Cary, N.C.) and the means were separated using Tukey's test ($P \leq 0.01$).

Conclusion

Due to our results, irradiation with 1.5 kGy improved the color and some quality indices of *A. bisporus* more than control and other gamma doses. Consequently, we can recommend the irradiation with suitable γ-ray dose in postharvest stage as a good practice to increase the shelf-life. Our results suggest irradiation also increased nutritive values by promoting production of antioxidants.

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