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Effect of Electron Beam Irradiation on Postharvest Quality and Selected Enzyme Activities of the White Button Mushroom, *Agaricus bisporus*

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Agaricus bisporus fruit bodies were exposed to different doses (1, 2, 3, and 4 kGy) of electron beam irradiation (EBI), and various physiological changes associated with postharvest deterioration, as well as selected enzymes considered to play a role in senescence, were monitored over a subsequent 16-day storage period at 4 °C and 75−85% relative humidity. EBI retarded postharvest mushroom softening and overall increases in malondialdehyde levels were more pronounced in controls compared with those of the irradiated samples. After 10 days of storage, polyphenoloxidase activity in samples irradiated with 1−4 kGy doses was significantly ($P < 0.05$) lower compared to that in control samples. Superoxide dismutase activity generally declined throughout the postharvest storage period in both irradiated and control samples, but no clear correlation between enzyme activity and EBI dosage was evident. Catalase activity decreased more slowly and to a lesser extent in fruit bodies exposed to 1 kGy compared with that in the controls and the other irradiated samples.

KEYWORDS: Electron beam irradiation; *Agaricus bisporus*; fruit body quality; mushrooms; postharvest senescence

INTRODUCTION

The white button mushroom, *Agaricus bisporus*, is widely recognized for its nutritional, organoleptic, and medicinal properties, and is highly popular with consumers (*1*−*3*). It is cultivated extensively worldwide, and currently accounts for ∼40% of the global mushroom production. In China, the production of *A. bisporus* increased more than 7-fold (from 180,500 to 1,330,400 tonnes) during 1997−2003, due largely to the breeding of superior strains and improvements in spawn production and cultivation technology (*4*). However, despite these major advances on the production side, *A. bisporus* has a limited shelf life due to postharvest senescence (*5*), and a major problem for growers is to maintain the postharvest quality of their products, especially in cases where they have to be transported over large distances before reaching the market place. Short shelf-lives have been identified as a major impediment to the development of the mushroom industry, and extending the postharvest storage period while retaining freshness remains a high priority for researchers. Several procedures directed at slowing down the rate of postharvest deterioration in fresh mushrooms have been assessed including chemical treatments (*6*), controlled atmosphere (CA) storage (*7*,*8*), refrigeration (*9*), coating (*10*), and cultivation using $CaCl_2$-containing irrigation water (*11*). However, all of the above have associated drawbacks including safety considerations, discoloration, production of off-flavors, and contamination with pathogenic microorganisms, and are unsuitable for use on an industrial scale.

Food processing employing different forms of irradiation is well established as a physical, nonthermal mode of food preservation (cold-pasteurization) that processes foods at or nearly at ambient temperature. Doses of γ-irradiation inhibited cap opening and browning, stalk elongation, reduced the level of microbial contamination, and generally extended the shelf life of mushrooms without noticeable effects on taste qualities (*12*, *13*). Gamma-irradiation alone and in combination with refrigeration has been shown to prolong shelf life through reducing moisture loss and improving color and appearance (*14*). Another form of irradiation, electron beam irradiation (EBI), is also known to be highly effective in reducing harmful bacteria in fruits, vegetables, and other foods while preserving the fresh taste, aroma, texture, wholesomeness, and nutritional content (*15*−*17*). However, microbial-based factors represent only part of the problem, and *A. bisporus*, in common with other mushrooms, deteriorates rapidly after harvesting due to endogenous biochemical processes that lead to water loss, browning and softening, bruising, stipe elongation and hollowing, and pileus expansion and splitting (*18*−*20*). Therefore, we have undertaken a study to assess if EBI represents an effective and viable method for maintaining the freshness and prolonging the shelf life of this commercially important mushroom. Our objectives were to determine the effects of different doses of EBI on selected physical (weight loss, browning, and softening), chemical (total sugar and malondialdehyde content), and biochemical (polyphenol oxidase, superoxide dismutase, and catalase) parameters during postharvest storage for 16 days at

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Table 1. Effect of EBI on the Firmness and Total Sugar Content of *A. bisporus* Fruit Bodies during Storage at 4 °C[a]

	postharvest storage time (days)					
treatment	1	4	7	10	13	16
control	16.2 ± 1.5	16.1 ± 1.5	15.7 ± 1.4	15.7 ± 1.4	15.5 ± 1.4	10.0 ± 1.5
	(49.3 ± 2.0)	(35.3 ± 4.0)	(34.6 ± 4.6)	(26.2 ± 4.1)	(21.4 ± 3.1)	(13.9 ± 3.2)
1 kGy	21.7 ± 1.4	21.3 ± 1.2	19.8 ± 1.4	18.8 ± 1.4	15.9 ± 1.5	14.5 ± 1.4
	(41.3 ± 0.8)	(27.4 ± 0.6)	(25.6 ± 0.1)	(17.9 ± 0.4)	(14.2 ± 0.9)	(15.6 ± 3.3)
2 kGy	22.4 ± 1.5	21.3 ± 1.4	20.1 ± 1.3	19.3 ± 1.5	18.9 ± 1.2	16.5 ± 1.4
	(45.2 ± 0.5)	(32.6 ± 0.3)	(27.1 ± 0.1)	(25.5 ± 0.9)	(20.1 ± 0.8)	(10.4 ± (1.2)
3 kGy	20.0 ± 1.5	18.6 ± 1.4	16.8 ± 1.3	16.7 ± 1.4	15.3 ± 1.4	15.0 ± 1.5
	(48.6 ± 1.4)	(32.5 ± 4.0)	(24.5 ± 0.2)	(22.3 ± (0.4)	(16.7 ± 0.6)	(7.0 ± (1.3)
4 kGy	21.5 ± 1.5	18.2 ± 1.4	17.4 ± 1.5	17.3 ± 1.5	14.1 ± 1.5	13.0 ± 1.4
	(50.1 ± 1.2)	(29.6 ± (0.9)	(16.3 ± 4.3)	(11.4 ± (4.0)	(10.8 ± 3.1)	(6.6 ± 2.1)

[a] Values not within parentheses are the means (n = 75) ± standard deviation of firmness determinations expressed in Newtons. Values within parentheses are the mean (n = 3) ± standard deviation of total sugar determinations expressed as % dry weight.

4 °C. To our knowledge, this represents the first study to use intact *A. bisporus* sporophores to determine the effects of EBI on factors widely considered to play a role in mushroom senescence.

MATERIALS AND METHODS

Mushroom Samples. Freshly harvested *A. bisporus* fruit bodies of good commercial quality, weighing between 35 and 40 g and with a cap diameter of 5.2−5.5 cm, were purchased from a commercial mushroom cultivation facility near Shanghai. Samples were uniformly white in color and without blemishes, and only mushrooms with intact veils were selected. Immediately after harvesting, fruit bodies were placed in open-sided plastic baskets (30 cm × 50 cm × 15 cm) and rapidly precooled for 4 h in on-site chilling facilities operated at 3 °C. Mushrooms were then packed in polystyrene trays (10 × 10 × 1 cm), covered with plastic film (PVC), and transported at 4 °C to the irradiation center of the Shanghai Academy of Agricultural Sciences (SAAS).

Electron Beam Irradiation. Electron beam irradiation was performed using an electron beam accelerator (ESS-010-03 electron linear accelerator). Mushroom trays were placed in a single layer in metal boxes on a conveyor and subjected to EBI at four different dosage levels of 1.0, 2.0, 3.0, and 4.0 kGy. A total of 48 trays were irradiated at each dose level. An untreated set of trays served as the control. After irradiation, the trays were stored in a cold chamber at 4 ± 0.5 °C and 75−85% relative humidity prior to analysis. After 24 h and subsequently every three days, samples in triplicate from each treatment group were randomly selected and analyzed as described below.

Total Sugar Determination. Total sugar in samples of powdered, freeze-dried *A. bisporus* fruit bodies were determined by the method of Dubois et al. (*21*).

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

$$\text{weight loss } (\%) = [(W_i - W_s)/W_i] \times 100$$

where W_i = initial weight and W_s = weight at sampling period.

Fruit Body Firmness. The firmness of *A. bisporus* fruit body pilei was measured using a TA.XT-plus texture analyzer (Stable Micro Systems Ltd., Godalming, U.K.) equipped with a P/5 probe. The probe was set at an initial height of 30 mm from the bottom of the holding plate to move at 2.0 mm/s. The maximum force (N) obtained during the test was recorded as the measurement of firmness. Tests were performed using 75 replicates for every treatment at each sampling time during the storage period. Firmness values are expressed in Newtons.

Fruit Body Extracts. Frozen *A. bisporus* tissue (1.0 g) was ground in a cold mortar and pestle with 10 mL of 0.05 mol/L phosphate buffer (pH 7.0) containing polyvinylpyrrolidone (0.1 g/mL) and EDTA (0.1 mol/L). Homogenates were centrifuged twice (12,000*g*, 15 min, 4 °C), and the supernatants were used for enzyme assays.

Enzyme Assays. *Superoxide Dismutase*. SOD activity was determined at 30 °C by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Constantine and Stanley with minor modifications (*22*). Reaction mixtures contained (in a total volume of 3 mL) 13 mM methionine, 0.075 mM NBT, 0.1 mM EDTA, 0.002 mM riboflavin, and 0.1 mL enzyme extract in 50 mM phosphate buffer (pH 7.8). One unit of SOD activity was defined as the amount of enzyme required to inhibit the initial rate of NBT photoreduction by 50%, and SOD activity values are presented as unit/h/g Fw (fresh weight) of mushroom.

Catalase. CAT activity was assayed at 30 °C according to the method of Kato and Shimizu (*23*) by measuring the initial rate of H_2O_2 disappearance at 240 nm. Assays were initiated by the addition of a 0.1 mL extract to 2.9 mL reaction mixtures containing 0.1 M sodium phosphate buffer (pH 7.0) and 2 mM H_2O_2. Enzyme activity was calculated using ε_{240} for H_2O_2 of 40 mM^{-1} cm^{-1}.

Polyphenol Oxidase. PPO activity was assayed by measuring the linear increase in absorbance at 410 nm and 30 °C as described by Galeazzi et al. (*24*) using catechol as the substrate. Reaction mixtures contained 2.0 mL of 50 mM phosphate buffer (pH 7.0), 2% (w/v) catechol, and 0.2 mL of extract added to initiate the reaction. One unit (U) of PPO activity was defined as the amount of enzyme catalyzing an increase in absorbance at 410 nm of 0.01/min, and PPO activity values are presented as U/min/g Fw of mushroom.

Malonaldehyde Assay. MDA was assayed by the method described by Heath et al. (*25*). The concentration (n mol/g of FW) of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} using the following formula: $(D_{532} - OD_{600}) \times 40/(0.155 \times FW)$.

Statistical Analysis. All determinations were carried out at least in triplicate and the data subjected to analysis using SPSS software (version 10.0). Differences between variables were tested for significance by one-way ANOVA. Significantly different means ($P \leq 0.05$) were separated by the Tukey test.

RESULTS AND DISCUSSION

Effect of Irradiation on Mushroom Weight Loss and Texture. Weight losses in controls and irradiated samples decreased in parallel during the experimental storage period and were limited to < 10% maximum of the original values (data not shown). Final weight losses recorded in control samples (5.9% average) were not significantly different ($P > 0.05$) from those observed in irradiated mushrooms (5.3−8.0% average). These values are slightly lower compared to the weight losses in nonirradiated (8.2% average) and γ-irradiated (8.3−9.7% average) *Hypsizygus marmoreus* fruit bodies over a 25 day postirradiation storage period (*26*).

All irradiated samples exhibited a gradual softening throughout the postirradiation storage period with samples exposed to 1.0, 2.0, and 3.0 kGy exhibiting the slowest softening rate (**Table 1**). Firmness values of control samples remained relatively stable over the first 13 days of storage but were significantly ($P <$ 0.05) lower compared with the values recorded for irradiated samples for most of the period. Control samples underwent major

softening during the last three days of storage, and at the end of the 16-day storage period, firmness values of irradiated samples were between 30 and 65% higher (**Table 1**).

Previously reported (*27*) firmness loss in mushrooms during storage has been attributed to cell wall degradation by bacterial enzymes and increased activity of endogenous autolysins (*28*). *Pseudomonas* degrades mushroom fruit bodies by breaking down the intracellular matrix and reducing the central vacuole, resulting in partially collapsed cells and the loss of turgor pressure (*29*).

Effect of EBI on the Total Sugar Content of *A. bisporus* Fruit Bodies during Storage at 4 °C. Total sugar concentrations in harvested plant products are reported to be important indicators of postharvest deterioration (*30*). Total and soluble sugar was earlier reported to be the major respiration substrate in *A. bisporus* during postharvest storage (*30*), and steady decreases in the total sugar content were previously reported in fruit bodies stored at 12 °C for 12 days but otherwise untreated (*31*). In this study, total sugar levels in both irradiated and nonirradiated *A. bisporus* fruit bodies exhibited a gradual decrease throughout the postharvest storage period. After 16 days, sugar levels in samples irradiated with 2.0, 3.0, and 4.0 kGy declined to 23.0, 14.4, and 13.2% of initial concentrations, respectively, compared to 37.8% in sporophores irradiated with 1.0 kGy and 28.2% in control samples (Table 2). These rates of decline in total sugar levels are much higher than those reported for *H. marmoreus* fruit bodies, where after 16 days postharvest storage, between 78−96% of initial concentrations remained in control samples and fruit bodies exposed to 0.8−2.0 kGy ^{60}Co-irradiation (*26*).

Effect of EBI on Malondialdehyde (MDA) Levels in *A. bisporus* Fruit Bodies during Storage at 4 °C. MDA is generally considered to be an indicator of lipid peroxidation (*32*), one outcome of which is a reduction in membrane integrity resulting in increased membrane leakage and enhanced cell senescence (*33*). MDA levels in irradiated *A. bisporus* fruit bodies fluctuated considerably throughout the storage period, although an overall increase was detected in all of these samples after 16 days (**Figure 3**). MDA levels in control samples increased gradually during the first 13 days of storage and then decreased. However, at the end of the storage period, MDA concentrations in these samples (41.2 nmol/g fresh wt) were significantly higher than those detected in the irradiated samples (21.7, 19.9, 25.7, and 30.6 nmol/g fresh wt for samples exposed to 1.0, 2.0, 3.0, and 4.0 kGy, respectively). Furthermore, on the basis of initial and final concentrations, the overall increase in MDA levels was much more pronounced in controls (64.1%) compared with those in the irradiated samples (9.6, 46.3, 38.2, and 31.9% for samples exposed to 1.0, 2.0, 3.0, and 4.0 kGy, respectively) (**Figure 1**). Large initial increases in the MDA content were previously recorded in nonirradiated *H. marmoreus* fruit bodies compared with ^{60}Co-irradiated samples (*26*). In that study, irradiation with 0.8 kGy was reported to be highly effective in preventing MDA accumulation during early postharvest storage (0−4 days).

Effect of EBI on Polyphenol Oxidase, Superoxide Dismutase, and Catalase Activities in *A. bisporus* Fruit Bodies during Storage at 4 °C. PPOs are a common feature of fungal cells and a major contributor to the browning of fresh white edible mushrooms (*34*). Browning is due to the oxidation of *o*-diphenols to produce *o*-quinones that polymerize to form brown products (*35*, *36*). However, our investigation revealed no evident correlation between the different EBI treatments and postharvest PPO activities, although after 10 days storage, PPO activity in samples irradiated with 1−4 kGy doses (25.6−45.9 U/g fresh wt) was significantly ($P < 0.05$) lower compared to that in control samples (53.4 U/g fresh wt) (**Figure 2**). During the first four days of storage, large increases (63.6−88.2%) in PPO activity were

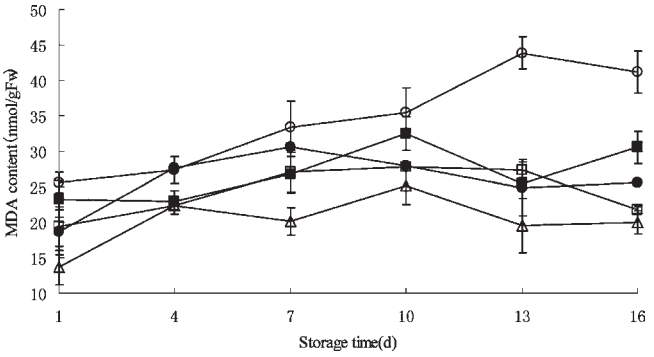

Figure 1. Effect of EBI on malondialdehyde levels in *A. bisporus* fruit bodies during storage at 4 °C. Control (○); 1.0 kGy (□); 2.0 kGy (△); 3.0 kGy (●); 4.0 kGy (■). Vertical bars represent the standard deviation about the mean ($n = 3$).

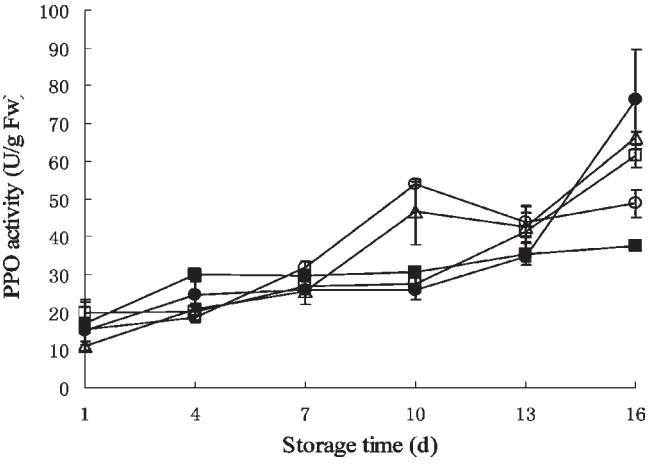

Figure 2. Effect of EBI on polyphenol oxidase activity in *A. bisporus* fruit bodies during storage at 4 °C. Control (○); 1.0 kGy (□); 2.0 kGy (△); 3.0 kGy (●); 4.0 kGy (■). Vertical bars represent the standard deviation about the mean ($n = 3$).

observed in samples exposed to 2, 3, and 4 kGy compared to only 18.4% in controls and ∼1.0% in fruit bodies treated with 1 kGy. However, whereas PPO activity in controls and samples exposed to 1 and 2 kGy continued (for the most part) to increase, enzyme activity in fruit bodies exposed to higher doses of irradiation remained constant for the next 7 days before increasing again toward the end of the storage period. PPO activity in control samples increased to peak values on day 10 (242% higher compared with day 1) before fluctuating at lower values during the last 6 days of storage (**Figure 2**). Koorapati et al. (*37*) reported that exposure to EBI at doses as high as 5.2 kGy did not affect the polyphenoloxidase activity in *A. bisporus* mushroom slices, whereas increases in PPO activity were recorded in whole *A. bisporus* fruit bodies γ-irradiated with 0.5, 1.0, and 2.0 kGy during the first 7, 9, and 12 days postharvest, respectively (*38*). Enzyme activity in *H. marmoreus* fruit bodies exposed to between 1.0 and 4.0 kGy of ^{60}Co-irradiation gradually increased in all samples during an initial 16−19 day postharvest period and then declined. Highest peak activity was recorded in nonirradiated controls, and peak activities in irradiated samples were inversely proportional to dosage.

SOD activity in both irradiated and control samples exhibited a general downward trend throughout the postharvest storage period, but again, there was no clear correlation between enzyme activity and the different EBI treatments (**Figure 3**). During the first four days of storage, changes in SOD activity were relatively

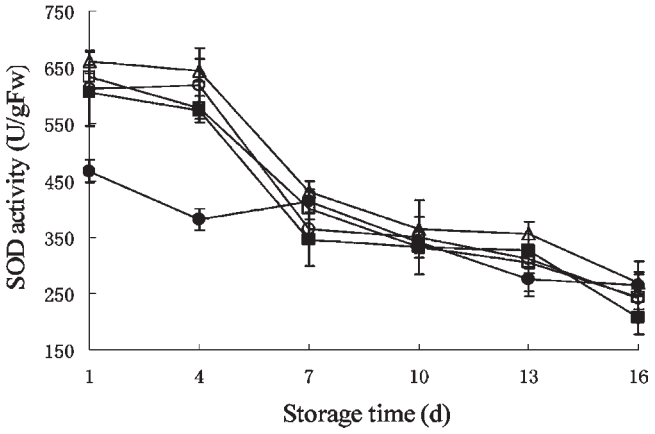

Figure 3. Effect of EBI on superoxide dismutase activity in *A. bisporus* fruit bodies during storage at 4 °C. Control (○); 1.0 kGy (□); 2.0 kGy (△); 3.0 kGy (●); 4.0 kGy (■). Vertical bars represent the standard deviation about the mean ($n = 3$).

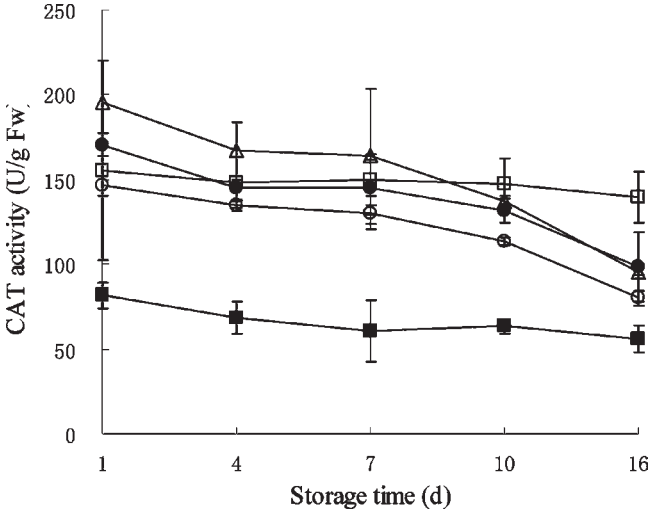

Figure 4. Effect of EBI on catalase activity in *A. bisporus* fruit bodies during storage at 4 °C. Control (○); 1.0 kGy (□); 2.0 kGy (△); 3.0 kGy (●); 4.0 kGy (■). Vertical bars represent the standard deviation about the mean ($n = 3$).

small (< 10%) in controls and fruit bodies irradiated with 1.0, 2.0, and 4.0 kGy, whereas samples exposed to 3.0 kGy recorded an 18.4% decrease. Conversely, the former samples exhibited large falls in enzyme activity (30.9−41.2% based on day 4 levels) during 4 to 7 days postirradiation treatment compared with a corresponding 8.7% increase in samples irradiated with 3.0 kGy. Similarly, the decline in SOD activity after 16 days of storage was significantly lower ($P < 0.05$) in samples exposed to 3.0 kGy (43.5%) compared to that in fruit bodies irradiated with 1.0 (61.8%), 2.0 (59.4%), and 4.0 kGy (65.8%), and controls (60.5%) (**Figure 3**).

Catalase activity in irradiated and control samples over a 16-day storage period is shown in **Figure 4**. Enzyme activity declined in all samples throughout the 16-day storage period, although considerable fluctuation in the rates of decline during individual 3-day sampling periods was observed, and in some cases, activities among the replicate samples varied appreciably (**Figure 4**). However, enzyme activity decreased at a significantly ($P < 0.05$) slower rate and to a lesser extent in fruit bodies irradiated with 1 kGy (10.3% during the entire sampling period) compared with corresponding decreases of 45.6%, 51.0%, 42.1%, and 32.7% for controls and samples exposed to 2.0, 3.0, and 4.0 kGy, respectively (**Figure 4**). Catalase activities in samples irradiated with 4 kGy were much lower throughout the sampling period (**Figure 4**).

Together, SOD (by converting superoxide anions into hydrogen peroxide) and CAT (by removing H_2O_2) serve to neutralize the destructive effects of reactive oxygen species on cellular components and are thought to alleviate postharvest deterioration by maintaining membrane integrity. SOD has been associated with stress tolerance in *A. bisporus*, and the gene encoding the enzyme is upregulated in postharvest sporophores (*39*). However, we were unable to detect any correlation between SOD and CAT activity and fruit body texture.

Food processing methods employing different forms of γ-irradiation are well established and have been used to preserve several edible mushrooms species. Doses of γ-irradiation inhibited cap opening, stalk elongation and browning, reduced the level of microbial contamination, and generally extended the shelf life of *A. bisporus*, with no noticeable effect on taste qualities (*12*, *13*). Furthermore, treatment of *Pleurotus nebrodensis* fruit bodies with 1.2 kGy γ-irradiation significantly delayed (by 6−9 days) the onset of fruit body softening, splitting, and browning compared with that in nonirradiated controls and test samples subjected to lower or higher irradiation doses (*40*). Exposure to 0.8 kGy γ-irradiation was clearly beneficial in maintaining the postharvest appearance of *H. marmoreus* sporophores compared to that in control samples (*26*). However, although EBI is known to be highly effective in reducing harmful bacteria in fruits, vegetables, and various meat-stuffs, there is a paucity of data relating to the use of this method to prolong the shelf life of freshly picked mushrooms. Koorapati et al. (*37*) evaluated the effects of EBI at dose levels of 0.5, 1, 3.1, and 5.2 kGy on *A. bisporus* mushroom slices and reported that EBI at 1 kGy was most effective in extending the shelf life. 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. Firmness of all samples was similar during storage except for the 5.2-kGy sample, and color was preserved by irradiation as evidenced by the higher L^* values. Our data increase the current understanding of the effects of EBI on the physiological and biochemical changes associated with postharvest senescence and should facilitate more targeted strategies for reducing postharvest quality loss in *A. bisporus* and other mushrooms.

In conclusion, our data indicate that exposure to appropriate doses of EBI has a clear beneficial effect in maintaining the postharvest texture of harvested *A. bisporus* fruit bodies. Overall, an irradiation dose of 2.0 kGy was marginally more effective compared with 1.0 and 3.0 kGy treatments in inhibiting the rate of softening throughout the 16-day sampling period. EBI also decreased the rate of MDA accumulation, and exposure to 1.0 kGy was particularly effective. The effects of irradiation treatment on PPO activity were less explicit, although smaller increases in enzyme activity were recorded during the first 10 days of storage in fruit bodies exposed to 1.0 kGy. Catalase activity decreased more slowly and to a lesser extent in fruit bodies exposed to 1 kGy compared with that in controls and the other irradiated samples.

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