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Effect of ^{60}Co-irradiation on Postharvest Quality and Selected Enzyme Activities of *Hypsizygus marmoreus* Fruit Bodies

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Hypsizygus marmoreus fruit bodies were exposed to different doses of ^{60}Co γ-irradiation, stored at 4 °C and 65–70% relative humidity, and various physiological changes associated with postharvest deterioration, as well as the activities of selected enzymes widely considered to play a role in the process of senescence, were monitored over a subsequent storage period of 25 days. Exposure to 0.8 kGy irradiation was clearly beneficial in maintaining the postharvest appearance of the mushroom sporophores compared to non-irradiated samples and fruit bodies exposed to higher doses (1.2–2.0 kGy) of irradiation. Samples treated with 0.8 kGy also exhibited smaller initial declines in soluble protein, smaller increases in reducing sugar content, and lower levels of malondialdehyde accumulation during the early storage period. Smallest increases in proteinase activity were recorded in samples dosed with 0.8 and 2.0 kGy, and levels of superoxide dismutase were significantly higher in samples exposed to 0.8 kGy compared with non-irradiated controls. Large initial increases in catalase activity were detected in samples irradiated with 0.8, 1.2, and 1.6 kGy and, although enzyme levels gradually decreased in all samples during further storage, residual levels after 25 days were still severalfold higher in irradiated samples compared with controls. The data increase the current understanding of the effects of γ-irradiation on the biochemical changes associated with postharvest senescence and should lead to more targeted strategies for reducing postharvest quality loss in *H. marmoreus* and other mushrooms.

KEYWORDS: ^{60}Co irradiation; fruit body quality; *Hypsizygus marmoreus*; mushrooms; postharvest senescence

INTRODUCTION

In 2002 (latest figures available), world production of cultivated mushrooms was estimated to have reached 12.25 million tonnes and was valued at approximately U.S. $32 billion (*1*). China, the world's main producer, contributed over 8.6 million tonnes (~70%). Because the cultivation of a particular different mushroom species is often confined to a few specific geographical locations within the country, a major problem for mushroom growers in China and elsewhere is to maintain the postharvest quality of their products that, in many cases, have to be transported over large distances before reaching the market place. Although cold-temperature storage is most often used to reduce postharvest deterioration, this method cannot be applied to all edible mushroom species. 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 (*2*). Doses of γ-irradiation inhibited cap opening, stalk elongation, and browning, reduced the level of microbial contamination, and generally extended the shelf life of the white button mushroom, *Agaricus bisporus*, with no noticeable affect on taste qualities (*3, 4*). γ-Irradiation also extended the storage life of *Pleurotus sajor-caju* (*5*) and *Pleurotus pulmonarius* (*6*) without affecting protein, amino acid, carbohydrate, and vitamin C contents. Ye et al. (*7*) and Liu et al. (*8*) reported that γ-irradiation of the straw mushroom, *Volvariella volvacea*, maintained membrane integrity, delayed cap opening and browning, and reduced the rates of decomposition and weight loss.

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The edible mushroom, *Hypsizygus marmoreus*, has long been recognized for both its organoleptic (*9*) and medicinal (*10*) properties. Wider appreciation of these attributes has led recently to a huge rise in consumer demand, which, combined with improvements in cultivation technology, has resulted in a dramatic increase in mushroom production. *H. marmoreus* fruit bodies, like those of other mushrooms such as *A. bisporus*, deteriorate rapidly after harvesting due to water loss, browning, and softening, stipe elongation and hollowing, and pileus expansion and splitting (*11–17*). However, in contrast to *A. bisporus*, low temperature storage is only partially effective in maintaining the postharvest quality of *H. marmoreus* because, after a few days at 4 °C, large numbers of aerial hyphae grow out from the surface of the pileus, greatly reducing consumer acceptability.

To our knowledge, there are no published data on the use of ^{60}Co γ-irradiation for enhancing the storage life of *H. marmoreus* fruit bodies. Furthermore, there is a lack of information on the physiological changes and biochemical reactions contributing to postharvest quality loss and their points of regulation. Therefore, we have studied the effects of different doses of irradiation on various physiological changes associated with postharvest deterioration, as well as on selected enzymes widely considered to play a role in the process of senescence. Our research is directed at retaining the economic value of harvested sporophores by providing an effective and viable method for improving the shelf life of this commercially important mushroom. Furthermore, because our data indicate that some biochemical features associated with postharvest senescence in mushrooms may be common to other foodstuffs, a better understanding of the effects of γ-irradiation on such changes could lead to more targeted strategies for reducing postharvest quality loss in other types of fresh agricultural produce.

MATERIALS AND METHODS

Mushroom Samples. Freshly harvested *H. marmoreus* fruit bodies of good commercial quality were obtained from Shanghai Finc Biotech Inc. Immediately after harvesting, fruit bodies were precooled, packaged in polystyrene trays (10 × 10 × 5 cm) covered with plastic film, stored at 4 °C, and transported to the irradiation center of the Shanghai Academy of Agricultural Sciences (SAAS).

Irradiation. Freshly harvested (up to 4 h) intact fruit bodies (140 g) were placed in plastic trays and irradiated at 20 °C with a ^{60}Co source at different dosage levels (0.8, 1.2, 1.6, and 2.0 kGy). The different irradiation doses were achieved by positioning the samples at different distances from the ^{60}Co source and exposing them to different dose rates as follows (irradiation dose, irradiation rate, irradiation distance): 0.8 kGy, 0.2 kGy/h, 215 cm; 1.2 kGy, 0.3 kGy/h, 175 cm; 1.6 kGy, 0.4 kGy/h, 150 cm; 2.0 kGy, 0.5 kGy/h, 130 cm). The total irradiation time was 4 h and the D_{max}/D_{min} ratio was 1.2. After 2 h of exposure, trays were inverted prior to irradiation for a further 2 h to provide more uniform treatment. Samples were then stored for 25 days at 4 ± 0.5 °C and 65–70% relative humidity. Forty-five replicates were included in each treatment group and, after 24 h and subsequently every 3 days, five replicates from each treatment group were randomly selected and sampled as described below.

Fruit Body Extracts. Frozen *H. marmoreus* tissue (1 g) was ground in a mortar and pestle with 10 mL of 0.05 M phosphate buffer (pH 7.0) containing 10% (w/v) polyvinylpyrrolidone (PVPP) and 0.1 M EDTA. Homogenates were centrifuged (15000*g*, 15 min, 4 °C) and supernatants used for enzyme assays.

Enzyme Assays. *Superoxide Dismutase.* SOD activity was determined by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Constantine and Stanley (*18*) 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 of 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 rate of NBT photoreduction by 50%, and SOD activity values are presented as units per hour per gram of fresh weight (FW) of mushroom.

Catalase. CAT activity was assayed according to the method of Kato and Shimizu (*19*) by measuring the initial rate of H_2O_2 disappearance at 240 nm. Assays were initiated by the addition of 0.1 mL of crude 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. (*20*) 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 units per minute per gram of FW of mushroom.

Proteinase. Proteinase activity was assayed using a modified version of the method described in ref *21* using dye-impregnated collagen (azocoll) as substrate. *H. marmoreus* fruit body extract (1.0 mL) was added to 3 mL of azocoll suspension (5 mg/ml) in shaken 50 mL flasks. After incubation at 30 °C for 1 h, residual azocoll was removed by centrifugation and the absorbance of the supernatant measured at 520 nm. One unit (U) of proteinase activity was defined as the amount of enzyme catalyzing an increase in absorbance at 520 nm of 0.01/h, and proteinase activity values are presented as units per hour per gram of FW of mushroom.

Soluble Protein. Soluble protein was determined according to the method of Bradford (*22*) using bovine serum albumin as standard.

Malonaldehyde Assay. MDA was measured essentially as described previously (*23*). Mushroom extract (1.0 mL) was mixed with 3.0 mL of 15% (w/v) trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid, and the mixture was heated at 100 °C for 18 min. After the sample had been cooled quickly to room temperature and centrifuged at 10000*g* for 10 min at 25 °C, the absorbance of the supernatant was measured at both 532 and 600 nm. The concentration (nanomoles per gram of FW) of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} using the following formula: $(OD^{532} - OD_{600} \times 40)/(0.155 \times FW)$.

Electrolyte Leakage Rate. Electrolyte leakage rate was measured essentially as described by Autio et al. (*24*). *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.

Reducing and Total Sugar. Reducing sugar and total sugar in samples of powdered, freeze-dried *H. marmoreus* fruit bodies were determined according to the methods of Miller (*25*) and Dubois et al. (*26*), respectively.

Fruit Body Firmness. The firmness of *H. marmoreus* fruit bodies was calculated from the first force peak on a Texture Profile Analysis (TPA) curve obtained using a TA.XT-plus texture analyzer (Stable Micro Systems Ltd., Godalming, U.K.) fitted with a HDP/BSK blade set. Tests were carried out using 90 replicates (one mushroom pileus = one replicate) at each sampling time during the storage period. The operating parameters were as follows: test mode, compression; pretest speed, 2 mm/s; test speed, 2 mm/s; post-test speed, 10 mm/s; target mode, 10 mm distant from probe; trigger type, 5.0 g auto force. Firmness values are expressed in newtons.

Aerial Hyphae Formation on Fruit Bodies. The incidence and extent of aerial hyphal growth was assessed daily by visual examination of 10 replicates of each treatment and scored as follows: 1, negligible, <5%; 2, slight, 5–10%; 3, moderate, 10–30%; 4, severe, >30%. Each sample was assigned a hyphal index calculated from Σ(hyphae score × percentage of fruit bodies with corresponding score).

Statistical Analysis. All extractions and determinations were carried out at least in triplicate, and the data were subjected to analysis of

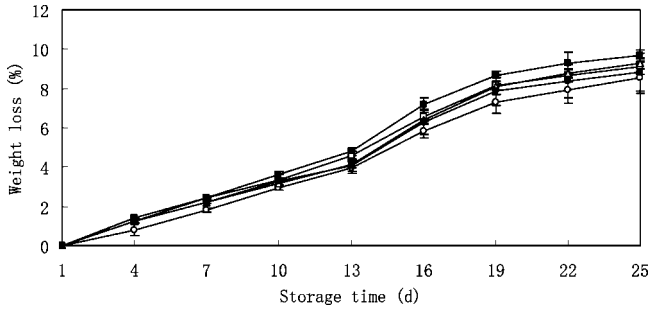

Figure 1. Effect of irradiation on weight loss by *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$).

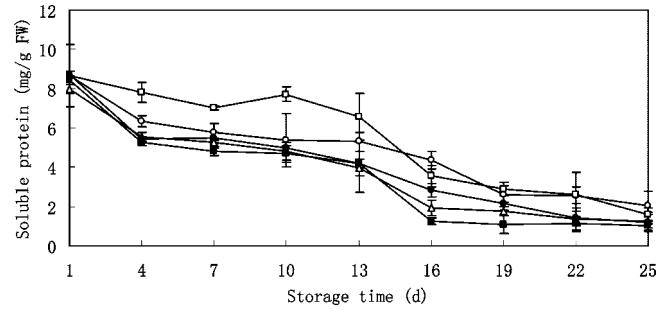

Figure 2. Effect of irradiation on the soluble protein content of *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

variance using DPS (version 7.05). Duncan's multiple-range test was employed to determine the statistical significance ($P = 0.05$) of the differences between the means.

RESULTS AND DISCUSSION

Effect of Irradiation on Overall Mushroom Quality. During the first 10 days of storage, virtually all fruit body samples exhibited small increases in firmness (from 16.5–18 to 19–21 newton units), although the differences between control samples and those exposed to the various irradiation treatments were not significant ($P > 0.05$) ($n = 95$). During subsequent storage, softening was observed in all samples to firmness levels recorded at the beginning of the experiment, but differences between controls and treatment samples were again insignificant, even at the highest irradiation dosage. Previous excessive softening induced in fruits and vegetables by exposure to 3 kGy of ionizing irradiation (*2*) was not observed, although the maximum dosage used in this study was only 2 kGy.

The appearance of aerial hyphae on *H. marmoreus* sporophores during low-temperature storage has a severe adverse effect on fruit body appearance and consumer acceptability. Increasing doses of irradiation delayed the appearance of hyphae in a dose-dependent manner. Extensive aerial hyphae were first evident on controls after 6 days of storage at 4 °C, whereas appearance was delayed until the 8th, 9th, 11th, and 12th day in samples exposed to 0.8, 1.2, 1.6, and 2.0 kGy irradiation, respectively. Moreover, the initial concentration of aerial hyphae was lower, and the subsequent rate of hyphal development on further storage was slower, in irradiated samples.

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 (**Figure 1**). Final weight losses recorded in controls (8.2% average) were not significantly different ($P > 0.05$) from those observed in irradiated samples (8.3–9.7% average).

Effect of Irradiation on Soluble Protein, Total Sugar, and Reducing Sugar Content of *H. marmoreus* Fruit Bodies during Storage at 4 °C. Fruit bodies of *H. marmoreus* contain substantial amounts of soluble protein (8.0–8.7 mg/g of FW) and, after harvesting, these serve as a nutrient source to support continuing metabolic activity. A decline in soluble protein concentration is considered to be an important indicator of tissue senescence (*27*). Soluble protein levels declined for all treatments during the 25-day post-irradiation period (**Figure 2**). However, the decline in soluble protein levels during the first 13 days in fruit bodies exposed to 0.8 kGy (24.1% of initial levels) was smaller compared with non-irradiated controls (38.4% of initial levels), whereas, at higher irradiation dose levels, the rate of decline during this period increased (~50% of initial levels) (**Figure 1**). Irradiated samples and, to a lesser extent, non-irradiated controls exhibited relatively large decreases in soluble protein content between 13 and 16 days post-treatment compared with the previous 9-day postharvest period. After 25 days of storage, residual soluble protein levels ranged between 18.5 and 12% of initial values compared with 23% in non-irradiated controls.

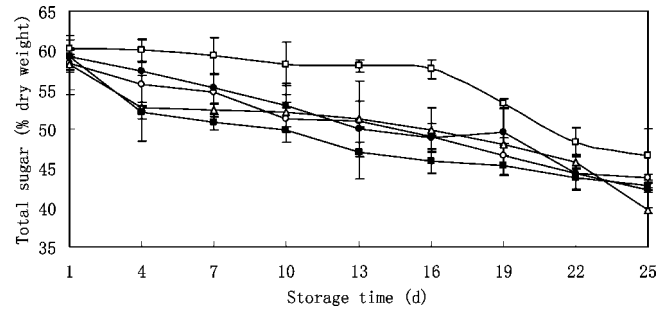

Figure 3. Effect of irradiation on the total sugar content of *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

Total and soluble sugar concentrations in harvested plant products are also considered important indicators of postharvest deterioration (*28*) Total sugar levels in non-irradiated *H. marmoreus* fruit bodies decreased at a rate not significantly different ($P < 0.05$) from those exposed to 1.2, 1.6, and 2.0 kGy over the 25-day post-irradiation period, and 68–75% of initial concentrations remained at the termination of the experiment (**Figure 3**). However, there was a much slower decline in the sugar content of sporophores irradiated with 0.8 kGy during the first 16 days following irradiation, and 96% of the initial carbohydrate remained compared with 78–86% in the other treatments. Steady decreases in the total sugar content were also reported in *Agaricus bisporus* mushrooms stored at 12 °C for 12 days (*29*).

Reducing sugar levels increased in all samples during the 25-day storage period (**Figure 4**). Although the rate of increase was initially generally constant in both irradiated samples and non-irradiated controls, levels increased at an accelerated rate between 13 and 16 days and between 16 and 19 days postharvest in samples irradiated with 2.0 and 1.6 kGy, respectively. A smaller acceleration was observed between 10 and 13 days postharvest in controls and samples exposed to doses of 1.2 kGy (**Figure 4**). Total increases in reducing sugar in these samples ranged from 124 to 137%. In contrast, relatively small increases in the reducing sugar content of samples treated with

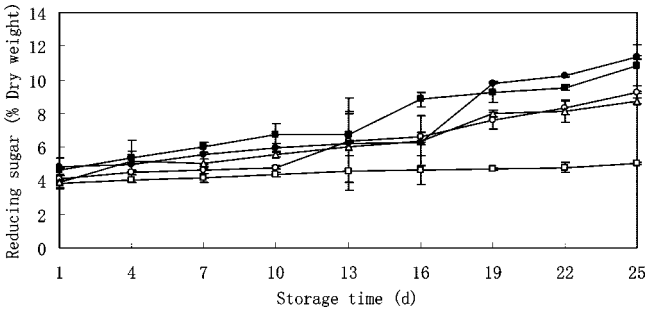

Figure 4. Effect of irradiation on the reducing sugar content of *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

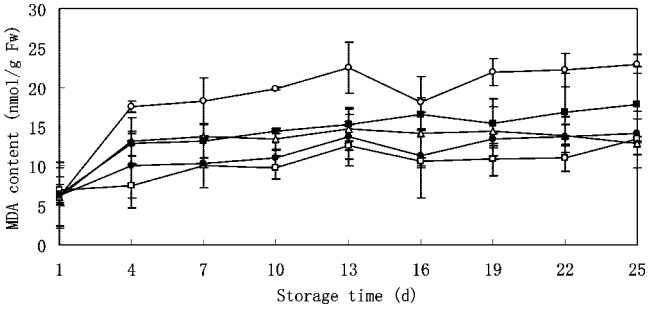

Figure 5. Effect of irradiation on the MDA content of *H. marmoreus* fruit bodies during storage at 4 °C.: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

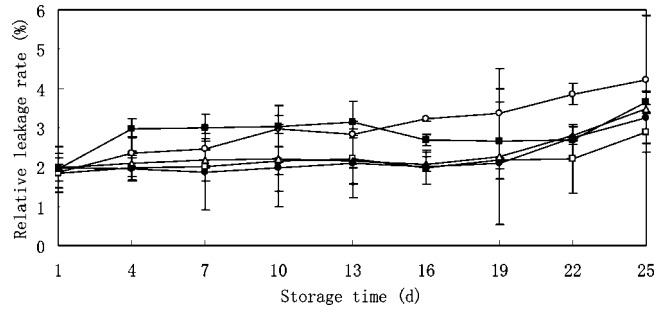

Figure 6. Effect of irradiation on the leakage of electrolytes from *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

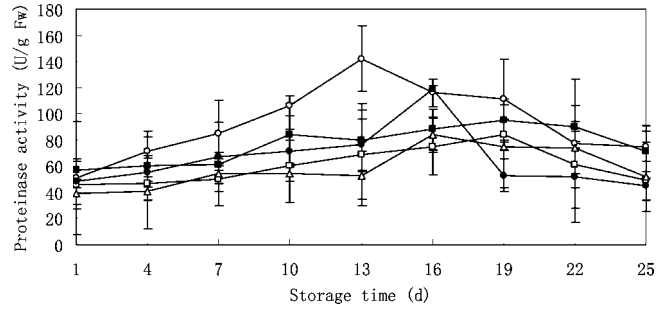

Figure 7. Effect of irradiation on proteinase activity in *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

0.8 kGy were recorded. Levels remained constant throughout the postharvest period and were only 32% higher than initial levels when the experiment was terminated.

Effect of Irradiation on Malondialdehyde (MDA) Levels in *H. marmoreus* Fruit Bodies during Storage at 4 °C. MDA is widely applied as an index of lipid peroxidation and rancidity in various foods, especially meat and fish products (*30*). The effects of postharvest irradiation on MDA levels in *H. marmoreus* fruit bodies during storage at 4 °C are shown in **Figure 5**. MDA levels in non-irradiated controls increased almost 2-fold during the first 4 days of the postharvest period. Large increases were also observed in fruit bodies irradiated with 1.2 kGy (116%), 1.6 kGy (60%), and 2.0 kGy (103%). However, MDA levels in samples irradiated with 0.8 kGy increased by only 7% during this period. Furthermore, more gradual increases in the MDA levels of both irradiated samples and controls were recorded during the next 21 days. When the experiment was terminated, MDA levels in controls and samples dosed with 2.0 kGy were 383 and 280% higher than immediate postharvest levels. Corresponding values for samples irradiated with 0.8, 1.2, and 1.6 kGy were 193, 216, and 226%, respectively (**Figure 5**).

Effect of Irradiation on Electrolyte Leakage Rates in *H. marmoreus* Fruit Bodies during Storage at 4 °C. Rates of electrolyte leakage from *H. marmoreus* fruit bodies remained constant during the first 19 days of storage in samples exposed to irradiation doses of 0.8, 1.2, and 1.6 kGy, whereas relative electrolyte leakage values for non-irradiated controls showed significant increases ($P < 0.05$) during this period (**Figure 6**). After 19 days of storage, electrolyte leakage increased at similar rates in both the irradiated and control samples. Leakage in samples irradiated with 2 kGy increased during the first 4 days after exposure, remained constant for the following 9 days, and then fluctuated irregularly (**Figure 6**).

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 (*31*). Therefore, the close overall correlation between changes in MDA levels resulting from peroxidation of membrane lipids and electrolyte leakage rates among the various samples (cf. **Figures 5** and **6**) is unsurprising. These processes clearly play an important role in postharvest deterioration because decreased rates of membrane lipid peroxidation and membrane leakage observed following treatment of *V. volvacea* and *L. edodes* fruit bodies with ^{60}Co irradiation (*7*) and calcium chloride (*32*), respectively, were accompanied by marked prolongation of postharvest mushroom freshness.

Effect of Irradiation on Proteinase, Superoxide Dismutase (SOD), Polyphenol Oxidase, and Catalase Activities in *H. marmoreus* Fruit Bodies during Storage at 4 °C. Proteinase activity in non-irradiated controls increased by 280% during the first 13 days of postharvest storage before declining to approximately half the recorded peak levels after 25 days (**Figure 7**). Enzyme activity in irradiated samples also increased, although peak levels were recorded later (between 16 and 19 days) and were lower than controls. The smallest increases in proteinase activity occurred in samples dosed with 0.8 kGy (183%) and 2.0 kGy (167%), respectively (**Figure 7**).

Gradual increases in polyphenol oxidase activity were observed in both irradiated samples and non-irradiated controls during the first 13–16 days postharvest, although enzyme levels in the latter were between 17 and 43% higher compared with irradiated fruit bodies during this period (**Figure 8**). Sharper rises to peak values occurred at day 16 in controls and at day 19 in irradiated material, and peak activity in controls was 154% higher compared with the lowest peak levels recorded in samples irradiated with 0.8 kGy (**Figure 8**).

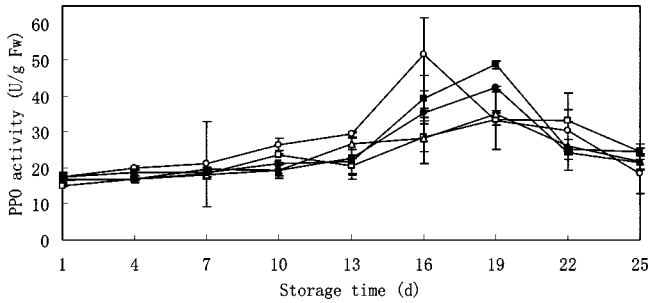

Figure 8. Effect of irradiation on polyphenol oxidase activity in *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

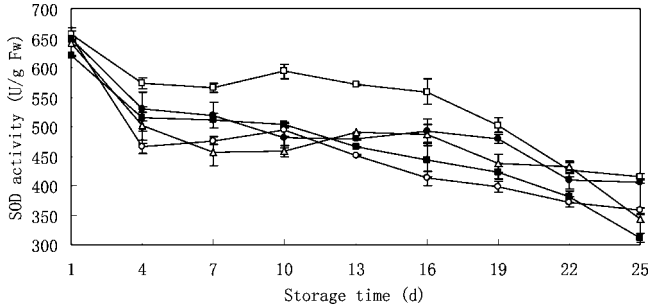

Figure 9. Effect of irradiation on superoxide dismutase activity in *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Standard deviation about the mean <10% ($n = 3$).

Time courses of SOD activity in irradiated samples and non-irradiated controls are shown in **Figure 9**. After 4 days of postharvest storage, a relatively large decrease in enzyme activity was recorded in controls (29%) compared with samples exposed to 0.8 (13%), 1.2 (22%), 1.6 (19%), and 2.0 kGy (17%). SOD levels then exhibited a gradual downward trend over the next 24 days, after which time 63% of the original levels remained in samples irradiated with 0.8 and 1.6 kGy compared with 55% in controls. Enzyme activity in samples exposed to 0.8 kGy was significantly higher than in controls ($P < 0.05$) during the first 19 days of the postharvest storage period (**Figure 9**).

Large increases in catalase activity were recorded in samples irradiated with 0.8 (79%), 1.2 (85%), and 1.6 kGy (61%) during the first 4 days of the postharvest storage period, and markedly higher initial enzyme activities were present in samples irradiated with 0.8 (58.8 units/mL) and 1.6 kGy (62.4 units/mL) (**Figure 10**). Catalase activity in controls gradually decreased throughout the postharvest period from an initial level of approximately 24 to 3 units/mL after 24 days. Although enzyme levels in irradiated samples also decreased during further postharvest storage, residual levels were still relatively high (18–40 units/mL) at the termination of the experiment (**Figure 10**).

Proteinases have earlier been implicated as playing a key role in the postharvest deterioration of edible mushrooms including *A. bisporus* (*27*, *33*, *34*), *V. volvacea* (*35*), and *Pleurotus* spp. (*35*, *36*). Burton (*33*) reported a 12-fold increase in proteinase activity in parts of *A. bisporus* pilei after harvesting that was not seen in mushrooms continuing to develop in contact with their mycelium. Metallo- and serine-proteinases were shown to be the predominant proteinases (*37*), and accumulation of a serine proteinase in senescent sporophores of *A. bisporus* (*27*), and increased postharvest expression of serine-proteinase

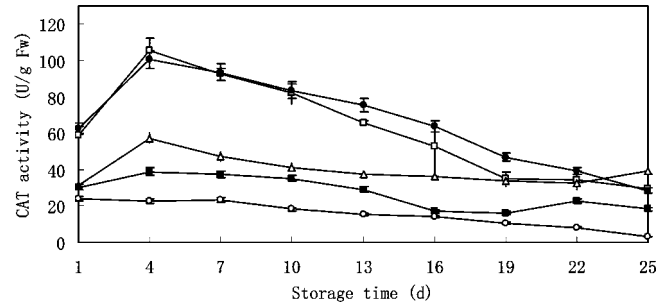

Figure 10. Effect of irradiation on catalase activity in *H. marmoreus* fruit bodies during storage at 4 °C: control (○); 0.8 kGy (□); 1.2 kGy (△); 1.6 kGy (●); 2.0 kGy (■). Vertical bars represent standard deviation about the mean ($n = 3$); no bars indicate SD <10%.

transcripts (*34*) was subsequently reported. Delaying the onset of postharvest peak proteinase activity in *V. volvacea* and *Pleurotus cornucopiae* using different temperature treatments also prolonged mushroom freshness (*35*).

Polyphenol oxidases are widespread in fungus cells and are the most important factor in the browning of fresh white edible fungi. Browning is due to the oxidation of *o*-diphenols to produce *o*-quinones that polymerize to form brown products (*38*, *39*).

Benoit et al. (*40*) reported increases in PPO activity until days 7, 9, and 12 for *A. bisporus* fruit bodies treated with 0.5, 1.0, and 2.0 kGy, respectively. The strain of *H. marmoreus* used in this study produced gray-brown fruit bodies, and browning was not a major concern. However, our data are expected to have major implications for the postharvest treatment of white strains of this mushroom that have been developed recently and which are rapidly becoming increasingly popular with consumers.

SOD and catalase protect cells from the damaging effects of reactive oxygen species and represent key components of cellular antioxidant defense systems. SOD catalyzes the dismutation of superoxide anions to produce hydrogen peroxide, which is then removed by catalase, and the two enzymes are thought to extend food freshness by protecting the integrity of membranes. In *A. bisporus*, SOD has been associated with stress tolerance, and the gene encoding the enzyme is reported to be up-regulated in postharvest sporophores.

The effects of irradiation on various physicochemical and biochemical characteristics of *H. marmoreus* fruit bodies during storage at 4 °C are summarized in **Table 1**.

World production of cultivated mushrooms during the period 1989–2002 is estimated to have increased >3-fold due largely to a wider recognition of their nutritional, organoleptic, and health-promoting qualities. However, although the industry has witnessed major advances in cultivation technology and strain improvement, short shelf life is still a major problem facing mushroom producers in terms of distribution and marketing of the fresh products, and extending the postharvest storage period remains a high priority.

Our data show that exposure to irradiation had a clear beneficial effect on the postharvest appearance of harvested *H. marmoreus* fruit bodies and on various biochemical activities previously linked to postharvest deterioration compared to non-irradiated samples. In the case of several parameters, exposure of sporophores to 0.8 kGy produced greater beneficial effects compared to higher doses of irradiation, whereas positive effects on other features were either dose-related or apparently dose-independent.

Although there is still consumer resistance to food irradiation, research in the United States and elsewhere has confirmed the safety and wholesomeness of foods irradiated under Good

Table 1. Summary of Effects of Irradiation on Various Physicochemical and Biochemical Characteristics of *H. marmoreus* Fruit Bodies during Storage at 4 °C

parameter	summarized observations
firmness	no significant difference between irradiated samples and non-irradiated controls
aerial hyphae	delay in appearance directly related to irradiation dosage; initial concentration and subsequent rate of development slower in irradiated samples
weight loss	no significant difference between irradiated samples and non-irradiated controls.
soluble protein	decreased initially (0–13 days) at a lower rate in 0.8 kGy irradiated samples, and at a higher rate in other irradiated samples, compared with controls; after 25 days, similar residual levels were recorded in controls and 0.8 kGy irradiated samples; residual levels in other irradiated samples were significantly lower.
total sugar	rates of decrease not significantly different in samples irradiated with 1.2, 1.6, and 2.0 kGy compared with controls; significantly slower rate of decrease in 0.8 kGy irradiated samples.
reducing sugar	increased levels (124–137%) recorded in controls and samples irradiated with 1.2, 1.6, and 2.0 kGy compared with only a 32% increase in 0.8 kGy irradiated samples
electrolyte leakage	exposure to all doses of irradiation decreased electrolyte leakage rates compared with controls, although increased leakage rates were recorded in 2.0 kGy irradiated samples during initial 13 day storage period
malondialdehyde (MDA) content	large initial increase in MDA content of controls compared with irradiated samples; irradiation with 0.8 kGy highly effective in preventing MDA accumulation in early storage period (0–4 days)
proteinase	enzyme levels initially increased in all samples, although peak values were much lower and occurred later in irradiated samples compared with controls; smallest increases were recorded in samples dosed with 0.8 and 2.0 kGy
polyphenoloxidase	enzyme levels gradually increased in all samples during initial 16–19 day period and then declined; highest peak activity was recorded in controls; peak activities in irradiated samples were inversely proportional to dosage
superoxide dismutase	relatively large decrease in enzyme levels in controls compared with irradiated samples during first 4 days of storage; levels then remained relatively stable for next 9–12 days and then decreased further; levels in 0.8 kGy irradiated samples were much higher than in controls during first 19 days of storage
catalase	higher initial enzyme levels recorded in 0.8 and 1.6 kGy irradiated samples; large increases detected in these and 1.2 kGy irradiated samples after 4 days of storage; enzyme levels gradually decreased in all samples during further storage; after 25 days, residual levels were severalfold higher in irradiated samples compared with controls

Manufacturing Practices. It is our belief that irradiation as a method of prolonging the shelf life of *H. marmoreus* warrants further study, and research is now underway in our laboratory to determine the effects of irradiation treatment on the nutritional (e.g., protein, amino acid, carbohydrate, and vitamin contents), organoleptic (e.g., texture, aroma), and microbiological qualities of this mushroom.

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