Dose Rate Effect of γ Irradiation on Phenolic Compounds, Polyphenol Oxidase, and Browning of Mushrooms (*Agaricus bisporus*)

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To enhance the shelf life of edible mature mushrooms, *Agaricus bisporus*, 2 kGy ionizing treatments were applied at two different dose rates: 4.5 kGy/h (I^-) and 32 kGy/h (I^+). Both I^+ and I^- showed a 2 and 4 day shelf-life enhancement compared to the control (C). Before day 9, no significant difference ($p > 0.05$) in L^* value was detected in irradiated mushrooms. However, after day 9, the highest observed L^* value (whiteness) was obtained for the mushrooms irradiated in I^-. Analyses of phenolic compounds revealed that mushrooms in I^- contained more phenols than I^+ and C, the latter containing the lower level of phenols. The fluctuation of the precursors of glutaminyl-4-hydroxyaniline (GHB) was less in I^- than in I^+. The polyphenol oxidase (PPO) activities of irradiated mushrooms, analyzed via catechol oxidase, dopa oxidase, and tyrosine hydroxylase substrates, were found to be significantly lowered ($p = 0.05$) compared to C, with a further decrease in I^+. Analyses of the enzymes indicated that PPO activity was lower in I^+, contrasting with its lower phenols concentration. The observation of mushrooms' cellular membranes, by electronic microscopy, revealed a better preserved integrity in I^- than in I^+. It is thus assumed that the browning effect observed in I^+ was caused by both the decompartmentation of vacuolar phenol and the entry of molecular oxygen into the cell cytoplasm. The synergetic effect of the residual active PPO and the molecular oxygen, in contact with the phenols, allowed an increased oxidation rate and, therefore, a more pronounced browning I^+ than in I^-.

Keywords: *Agaricus bisporus; browning; polyphenol oxidase (PPO); phenols; γ irradiation.*

INTRODUCTION

Browning reaction in fruits and vegetables is recognized as a serious problem in the food industry. The browning of mushrooms upon storage is a rather complex process (MacCanna and Garmley, 1968). Indeed, the mushroom cells cause browning when they are subjected to forces that can disrupt cellular integrity, such as vibrations, rough handling, and aging (Guthrie, 1984; Hughes, 1958). The polyphenoloxidase (PPO) present in the pileus (cap) and stipe (stalk) of mushrooms plays also an important role. PPO is a copper-containing enzyme (Vámos-Vigyázó, 1981), which catalyzes two different reactions: (i) the hydroxylation of monophenols to the corresponding *o*-dihydroxy compounds and (ii) the oxidation of *o*-dihydroxy phenols to *o*-quinones, which condense to form the brown melanin pigments (Long and Alben, 1969; Stussi and Rast, 1981).

The principal phenolic compound in mushrooms is glutaminyl-4-hydroxyalanine (GHB) (Scheme 1). The PPO monophenolase-mediated hydroxylation of GHB produces glutaminyl-3,4-dihydroxybenzene (GDHB) and an iminoquinone that is the 2-electron-deficient form of GHB. In this reaction, **GHB** is reported to act both as the substrate and as a cofactor. The PPO diphenolase-mediated oxidation of GDHB produces the corresponding benzoquinone (GBQ) (Boekelheide et al., 1979). The latter yields melanin by polymerization (Stüssi and Rast, 1981) (Scheme 1). The control of PPO in mushrooms is desirable in light of the ban on use of sulfites to delay browning reactions.

The browning of mushrooms might also be caused by the action of bacteria and mold on the mushroom tissues. *Pseudomonas tolaasii* is regarded as a normal constituent of the microflora of the mushroom bed that could produce a metabolite toxic to mushrooms under certain conditions. The infection appears as a brown injury on mushrooms (Paine, 1919; Royse and Wuest, 1980). Molds can also affect the quality of the mushrooms. Contamination of *Verticillium maltousei* shows brown spots (Salunkle and Desai, 1984).

Some preservative methods could be used to keep the quality of mushrooms, such as low-dose γ irradiation. Several authors reported that γ irradiation at a dose of 2 kGy lowers the microorganism counts both initially and throughout the storage of mushrooms and, therefore, results in an increase of the shelf life from 2 to 10 days (Gautam et al., 1998; Beaulieu et al., 1992; Smierzchalska and Wojniakiewicz, 1986; Skou et al., 1974; Staden, 1966, 1967). However, at such a dose, the irradiation treatment causes oxidation of phenolic compounds present in vacuoles, which could induce a slight brownish discoloration of mushrooms (Kovács and Vas, 1974a; Thomas, 1988; Skou et al., 1974).

Most of the researchers agree that irradiated mushrooms retain their original skin color for longer periods or darken less rapidly than unirradiated mushrooms

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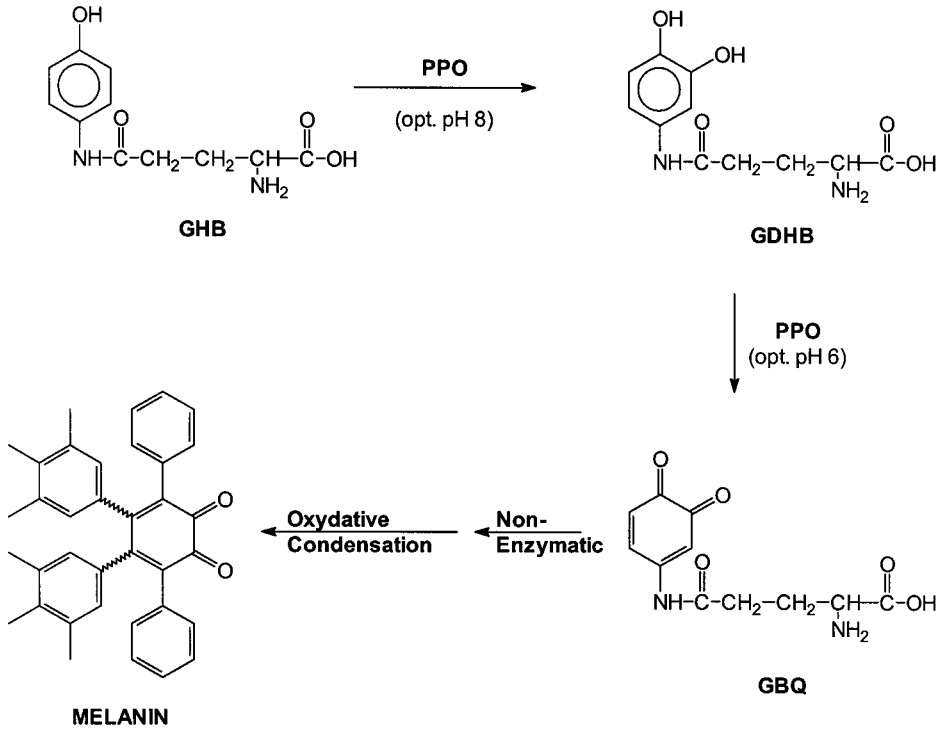
Scheme 1



(Kovács and Vas, 1974a; Thomas, 1988). The coloration change in mushrooms upon irradiation is still the subject of some controversies. Upon irradiation, a light browning effect, which seemed to be more apparent with accelerated electrons, was observed immediately after the treatment (Kovács and Vas, 1974a). However, Skou et al. (1974) did not observe any significant difference between the quality of mushrooms treated with accelerated electrons and mushrooms treated with γ rays. Gill et al. (1969) reported beneficial effects with a dose >0.5 kGy. Some authors observed a better preserved coloration with 1.0 kGy treatment (Kovács and Vas, 1974b; Kramer, 1986; Skou et al., 1974), whereas a 2.5 kGy dose was reported to be necessary to significantly decrease the browning effect (Roy and Balh, 1984). These studies were carried out in a ^{60}Co γ irradiation having a dose rate below 1 kGy/h.

A recent study (Beaulieu et al., 1992) reported that γ irradiation at low-dose rate (4.5 kGy/h) preserves the whiteness of the mushrooms, when compared to mushrooms treated at 32 kGy/h, and effectively lowers microorganism counts initially and throughout storage. Besides that study, none of the works presented so far in the literature had tackled the dose rate effect of the γ irradiation on edible mushrooms. We have, therefore, undertaken an in-depth investigation of the dose rate effect at 2 kGy treatment, namely, a low-dose rate, 4.5 kGy/h, and a high-dose rate, 32 kGy/h. The aim of this work was to learn and understand how the dose rate could delay and/or prevent the coloration change in mushrooms both upon storage and upon irradiation and, thus, increase their shelf life and decrease their loss during marketing. Several variables of nonirradiated and irradiated mushrooms were assessed during the 11 days of storage, among which were the color, the total phenolic compounds, the fate of different recognized GHB precursors, the activity of the PPO, and the mushroom cellular membranes.

MATERIALS AND METHODS

Samples. Thirty boxes (2 kg each) of edible mature mushrooms *A. bisporus* of the Albidus variety, 4−5 cm in diameter, from Excel producer (Montreal, PQ) were used for the experiments.

Irradiation Treatments. Mushrooms were irradiated (Canadian Irradiation Center, Laval, PQ) in triplicate at a dose of 2 kGy and at two dose rates: 4.5 and 32 kGy/h. The irradiation treatment at 32 kGy/h (I^+) was performed in an underwater calibrator (MDS Nordion International Inc., Kanata, ON). At a dose rate of 4.5 kGy/hr (I^-) an IJ 8900 carrier irradiator (MDS Nordion International Inc.) was employed. Both systems were equipped with ^{60}Co.

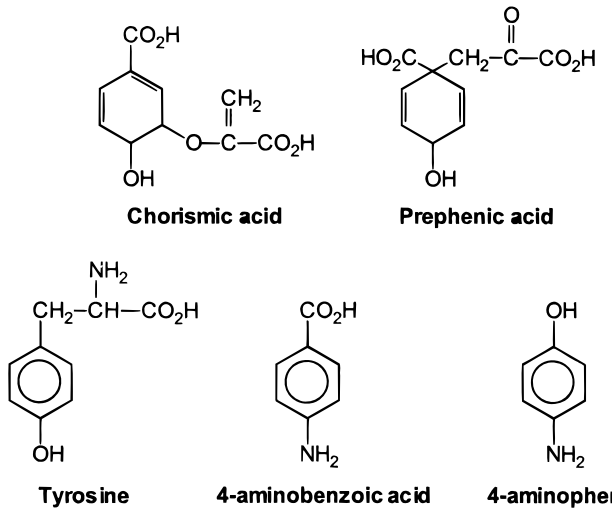
Storage Conditions. Treated (I^-, I^+) and control (C) mushrooms were stored at 15 °C in separate, sterile, and closed containers (26 L), with air flow filtered by active carbon and a 0.45 μm air filter. After filtration, the air was bubbled through sterile water to hold a 90% relative humidity in the box with an air rate of 78 L/h. This avoided the contamination of the mushrooms and stabilized the CO_2 concentration. Gas chromatography (Varian 3400 GC, 180 °C, He, high molecular seed column) analyses of CO_2, N_2, and O_2 revealed that their contents were identical to the ambient air.

Analyses. Mushroom analyses were performed on days 0, 2, 4, and 7 for C and on days 0, 2, 4, 7, 9, and 11 for both I^- and I^+ samples.

Color Measurement. The measurements of the mushroom cap coloration (hue) and whiteness (L^*) were performed with a Colormet colorimeter (Instrumar Ltd., St. John, NF) on 15 mushrooms per sample. This apparatus measures the light reflection spectrum and converts it to L^*, a^*, and b^* Cartesian coordinates of a three-dimensional space map of all colors (CIELAB 1976). The coordinates a^* and b^* were calculated as hue angle [$\tan^{-1}$ (b^*/a^*) and chroma [hue radius: $\sqrt{(a^{*2} + b^{*2})}$], representing, respectively, the color between the axis of yellow and red and the brightness of the color.

Phenol Content. The total phenolic compounds were extracted with ethanol and quantified after coloration with Folin−Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO) in a DMS 200 spectrophotometer (Varian Canada Inc.) at 760 nm (Marigo, 1973). The precursors of GHB (chorismic acid, prephenic acid, tyrosine, 4-aminobenzoic acid, and 4-amino-

Chart 1



phenol) (Chart 1) were determined after extraction in ethanol. Their separation and quantification were performed by HPLC (Vista 5500, autosampler 9090, Varian Canada Inc.) at 241 nm on a Particil 10-SCX column (Whatman Inc., Clifton, NJ) using a 5.0 mM sodium phosphate buffer pH gradient of 3.2−8.0 (Speroni and Beelman, 1982).

PPO Activity. The extractions of PPO were performed with acetone powder (Yamaguchi et al., 1970; Ingebrigtsen et al., 1989). The supernatant was portioned in two equal volumes to one of which was added 0.1% SDS (w/v) to determine the latent activity of PPO. The catechol oxidase, dopa oxidase, and tyrosine hydroxylase activities were respectively measured, using a Varian spectrophotometer, in oxygenated assay mixtures of 20 mM catechol, 16 mM L-dopa, and 1.5 mM l-tyrosine (Sigma Chemical Co.) in sodium phosphate buffer (pH 6.0) of 50, 50, and 7.5 mM, respectively. The respective formation rates (25 °C) of 3,4-benzoquinone, *o*-alanyl-3,4-benzoquinone, and 3,4-dihydroxyphenylalanine were measured in a Varian DMS 200 spectrophotometer at 410, 475, and 285 nm, respectively, every 0.1 s over a period of 3 min. The increase of one absorbance unit per minute defines one enzyme unit (IU).

Membrane Analyses. The mushroom cytoplasmic membrane structures were observed with a Philips EM 300 à 80 kV electronic transmission microscope following the method described by Athanassious and al. (1978).

Statistical Analyses. Results were submitted to an analysis of variance followed by Duncan multiple-range tests. The degree of confidence was fixed at 95% for all of the analyses (Snedecor and Cochran, 1978).

RESULTS

Color Measurement. During the storage, a decrease of the mushroom whiteness was observed for all mushrooms, control and treated (Figure 1). The *L** values in C decreased from 78 on day 0 to 67 on day 7. They decreased from 77 to 63 and from 76 to 56, respectively, in I^- and I^+, from day 0 to day 11 (Figure 1). Mushrooms in I^- are significantly ($p \leq 0.05$) darker than in I^+ and in C after 2 days of storage. The mushroom whiteness (*L** value) in C remained stable until day 4. However, after 4 days of storage a quick darkening was noticed until day 7. In I^- and I^+ this rapid loss of whiteness occurred from day 7 to day 11, when the value in I^- was significantly ($p \leq 0.05$) higher than in I^+ on day 11. After 7 days of storage, the *L** value in C was 67, whereas such a value was observed only after 9 days of storage in both I^- and I^+.

The color measurement revealed that the initial (day 0) mushroom cap hue value in C decreased from 84 to 79 on day 7 (Figure 2). In I^- and I^+ this value decreased, respectively, from 86 to 78 and from 84 to 76, from day 0 to day 11 (Figure 2). The hue value remained unchanged ($p > 0.05$) from day 0 to day 4 in the treated mushrooms, in which it decreased significantly from day 2 ($p \leq 0.05$) in C. No significant differences ($p > 0.05$) were observed between C and I^+. The cap hue value in C was 79 on day 7, whereas such a low hue was noticed after day 9 in both I^- and I^+. On day 11, the hue value in I^- was significantly higher than in I^+; that is, the mushroom caps were orange in I^+. These results demonstrate that the mushroom caps maintained a significantly yellower ($p \leq 0.05$) color in I^- throughout the storage, the yellow color being expressed by the high hue angle. The difference of the hue value is mainly due to a higher phenol oxidation in I^+ and C than in I^-. Indeed, as reported by Stussi and Rast (1981), GHB turns to orange upon oxidation (lower hue angle). To note, hue values depend also on the quality of the stipe, where PPO (the enzyme responsible for the phenol oxidation) concentration is higher (Moore et al., 1988).

On the basis of coloration, low-dose-rate irradiation delayed more effectively the mushroom browning, extending thus their shelf life by ∼4 days.

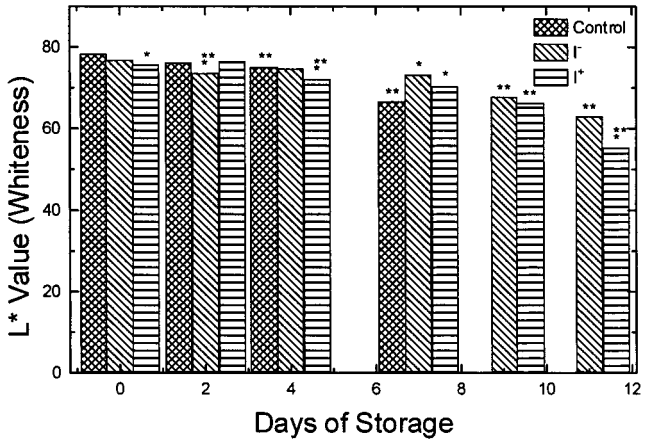


Figure 1. Mushroom browning during the storage period. A single star (*) at the top of a column indicates a significant difference ($p \leq 0.05$) within the same set of columns, at the same day; two stars (**) at the top of a column indicate a significant difference ($p \leq 0.05$) with the same column at the previous day of storage.

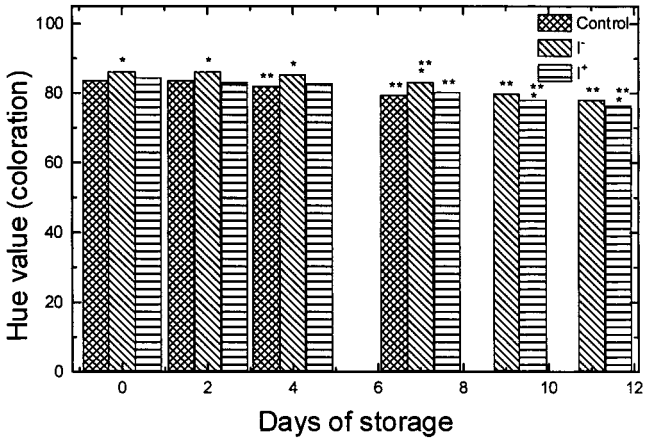


Figure 2. Mushroom coloration during the storage period. A single star (*) at the top of a column indicates a significant difference ($p \leq 0.05$) within the same set of columns, at the same day; two stars (**) at the top of a column indicate a significant difference ($p \leq 0.05$) with the same column at the previous day of storage.

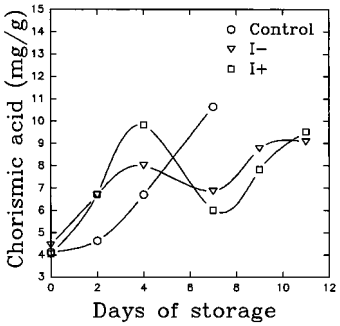


Figure 3. Fate of GHB precursor chorismic acid in mushrooms (mg/g of dry weight).

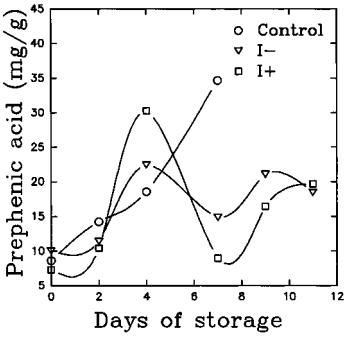


Figure 4. Fate of GHB precursor prephenic acid in mushrooms (mg/g of dry weight).

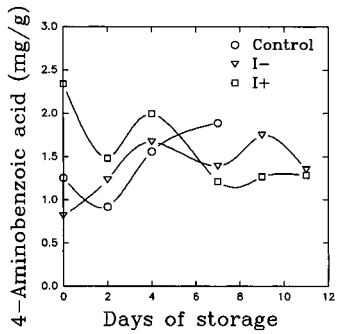


Figure 5. Fate of GHB precursor 4-aminobenzoic acid in mushrooms (mg/g of dry weight).

Phenol Content. Chorismic acid, prephenic acid, 4-aminobenzoic acid, and tyrosine are precursors to 4-aminophenol, itself a precursor to GHB in mushrooms (Boekelheide et al., 1979). The effects of both the irradiation at two different dose rates and the storage are shown in Figures 3−7.

Prephenic acid (Figure 4) and tyrosine (Figure 7) were found to be the two major compounds in mushrooms *A. bisporus*. Chorismic acid (Figure 3), prephenic acid (Figure 4), and 4-aminobenzoic acid (Figure 5) increased significantly ($p \leq 0.05$) in C during the storage. In I^+, the fluctuation of these compounds was greater than in I^- (Figures 3−5). The concentration of 4-aminophenol (Figure 6) was significantly ($p \leq 0.05$) greater in C than in either I^- or I^+ during storage. As the analysis of GHB content was not performed, it is difficult to define whether the 4-aminophenol found in I^- and I^+ was transformed to GHB and accumulated as is or whether it was transformed into other compounds.

During the storage period of the mushrooms, the content of total phenols (Figure 8) was significantly ($p \leq 0.05$) higher in I^- than in I^+ and C.

Analyses performed after the irradiation treatment indicated that irradiated mushrooms contained ~30% more phenols than control (Figure 8). The level of total phenols in C and in I^- (Figure 8) increased significantly ($p \leq 0.05$) between day 0 and day 2. After this period, the level of total phenols in C did not fluctuate, whereas the level of total phenols in I^- decreased significantly ($p \leq 0.05$) (Figure 8). At the end of the storage, the content of total phenols in I^- was not significantly ($p > 0.05$) different from the initial content (day 0). As for I^+, the irradiation did not affect significantly ($p > 0.05$) the concentration of total phenols. No significant difference was noticed between I^+ and C during days 2 and 11.

The greater phenol accumulation in I^- combined with its better preserved coloration indicates a lower oxidation rate in mushrooms irradiated at low irradiation dose rate.

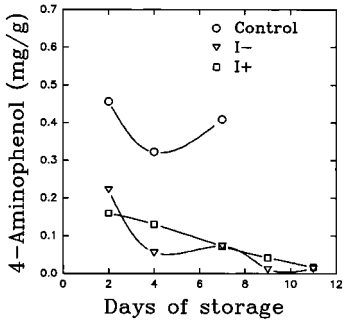


Figure 6. Fate of GHB precursor 4-aminophenol in mushrooms (mg/g of dry weight).

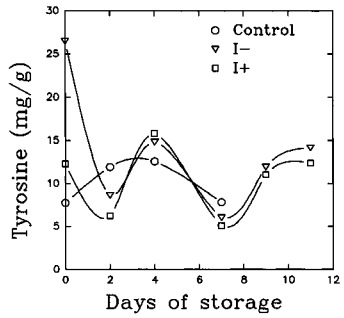


Figure 7. Fate of GHB precursor tyrosine in mushrooms (mg/g of dry weight).

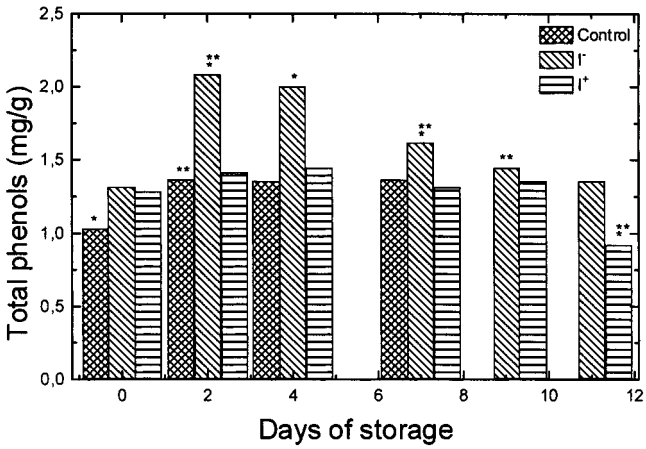


Figure 8. Fate of total phenolic compounds in mushrooms (mg/g dry weight). A single star (*) at the top of a column indicates a significant difference ($p \leq 0.05$) within the same set of columns, at the same day; two stars (**) at the top of a column indicate a significant difference ($p \leq 0.05$) with the same column at the previous day of storage.

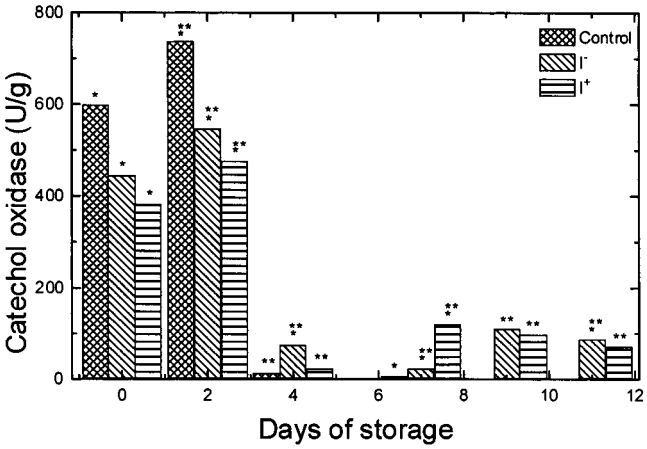


Figure 9. Mushroom PPO activity during the storage period (IU/g of fresh weight). A single star (*) at the top of a column indicates a significant difference ($p \leq 0.05$) within the same set of columns, at the same day; two stars (**) at the top of a column indicate a significant difference ($p \leq 0.05$) with the same column at the previous day of storage.

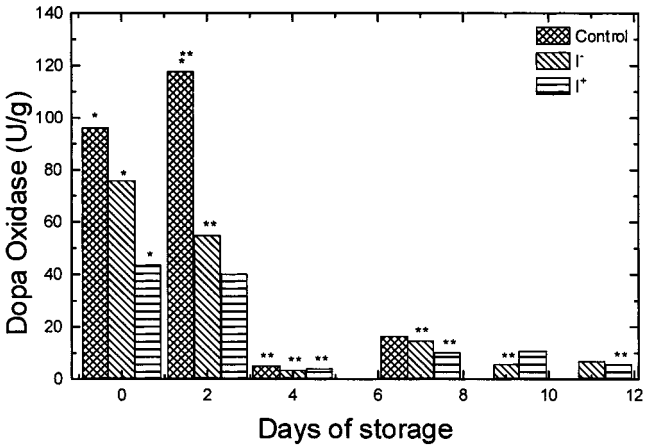


Figure 10. Mushroom PPO activity during the storage period (IU/g of fresh weight). A single star (*) at the top of a column indicates a significant difference ($p \leq 0.05$) within the same set of columns, at the same day; two stars (**) at the top of a column indicate a significant difference ($p \leq 0.05$) with the same column at the previous day of storage.

PPO Activity. The PPO activity was assessed by its catechol oxidase (Figure 9), dopa oxidase (Figure 10), and tyrosine hydroxylase (Figure 11) activities. In all cases, irradiated samples (I^- and I^+) exhibited a lower PPO activity compared to nonirradiated controls (C) (Figures 9−11). Other authors (Gautam et al., 1998) also observed a similar behavior. The PPO activity was found to be very high during the first 4 days of storage (Figures 9−11).

γ irradiation at 2 kGy at a low-dose rate (I^-) and a high-dose rate (I^+) resulted in a significant ($p \leq 0.05$) reduction of the catechol oxidase activity, with a further decrease in I^+ ($p \leq 0.05$) (Figure 9). During the storage, the catechol oxidase activity behaved similarly in C, I^-, and I^+, namely, a significant ($p \leq 0.05$) increase of the activity during the first 2 days of storage, followed by an important decrease from day 4 (Figure 9). On day 4 the activity in I^- was maintained higher ($p \leq 0.05$) with respect to both C and I^+. At day 4, C showed a very low catechol oxidase activity. Furthermore, from day 4, the catechol oxidase activity difference between C and I^-,I^+ was not as important as the first 2 days' activity (Figure 9). Although on day 7 the activity had significantly

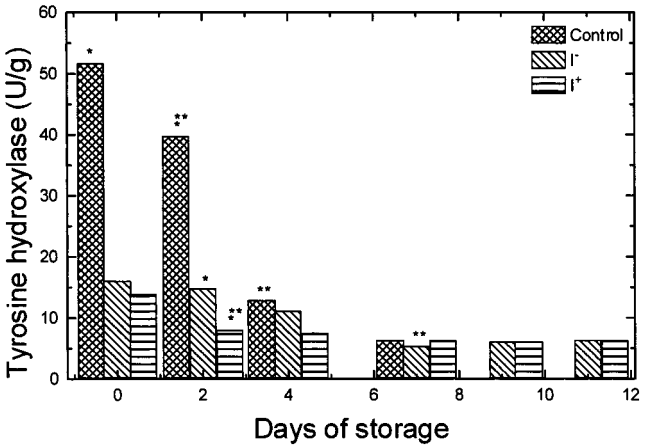


Figure 11. Mushroom PPO activity during the storage period (IU/g of fresh weight). A single star (*) at the top of a column indicates a significant difference ($p \leq 0.05$) within the same set of columns, at the same day; two stars (**) at the top of a column indicate a significant difference ($p \leq 0.05$) with the same column at the previous day of storage.

increased ($p \leq 0.05$) in I^+ and decreased ($p \leq 0.05$) in I^-, they were similar ($p > 0.05$) from day 9 to day 11. Globally, mushrooms in I^- had a higher activity than in I^+, except on day 7 (Figure 9).

The irradiation treatment had a similar effect on the dopa oxidase activity on day 0 (Figure 10). However, the dopa activity increased only in C between days 0 and 2, followed by an important decay (Figure 10). Irradiated mushrooms exhibited an enzymatic activity significantly ($p \leq 0.05$) lower than that of the controls (Figure 10). Moreover, the dopa activity decreased gradually and significantly ($p \leq 0.05$) during storage, from day 0 in I^- and from day 2 in I^+. Until day 4, the dopa oxidase activity in I^- was significantly ($p \leq 0.05$) higher with respect to I^+. After day 4, the dopa oxidase activity was not significantly different ($p > 0.05$) between C and I^-,I^+. Furthermore, from day 4 to day 11 the dopa oxidase activity was almost absent in all groups (Figure 10).

The tyrosine hydroxylase activity, on day 0, in I^- and I^+ showed a much greater reduction ($p \leq 0.05$) as compared to C (Figure 11). In fact, their activities on day 0 were similar to the activity reached on day 4 in C (Figure 11). Moreover, there was no significant difference ($p > 0.05$) between C, I^-, and I^+ from day 4 until the end of the storage (Figure 11). Likewise, the tyrosine hydroxylase activity in I^- was not significantly ($p > 0.05$) different from in I^+, except on day 2 (Figure 11). It shoudl be noted that the tyrosine hydroxylase activity was clearly lower than the catechol oxidase and dopa oxidase activities (Figures 9−11).

DISCUSSION

PPO was reported to be responsible for browning in mushrooms (Skou et al., 1974). The native PPO is a tetramer described as an H_2L_2 structure; the H subunits have molecular masses of 45−55 kDa, and the L subunits have molecular masses of 13−15 kDa (Robb, 1984), with no disulfide linkages between subunits (Robb et al., 1981). Each active site contains a pair of antiferromagnetically coupled cupric ions (Kempner and Miller, 1989). Referring to Figures 9−11, we notice that PPO activity in C is higher with respect to irradiated mushrooms, during the first 2 days of storage. Such

behavior is in agreement with results reported earlier (Gautam et al., 1998). This negative shift of PPO activity provoked by the irradiation could be due to a conformational change of the enzyme or to a modification of the active site, namely a reduction of the cupric ion of the enzyme (Fry and Strothkamp, 1983). This ion is required for oxidizing phenols.

The catechol and dopa oxidase activities measure the diphenol oxidase activity of PPO, which requires one H subunit (55 kDa) (Robb, 1984). The oxidation reaction caused by catechol oxidase is similar to the one caused by dopa oxidase. The difference between these two enzymes resides in the larger size and the dipolar isoelectric form of the dopa oxidase (Lehninger, 1982). Figures 9 and 10 clearly show that PPO has a lower activity when the dopa was used as substrate with respect to catechol oxidase. Moore and Flurkey (1989) also reported such an observation. It is assumed that the dopa oxidase is more sensitive to conformational change of PPO owing to its charge. As for the dose rate effect, the PPO activity in I^- and I^+ exhibited a reduction when catechol was the substrate, as compared to C on day 0. However, this reduction was more pronounced in I^+ than in I^- when dopa was the substrate. Yamaguchi et al. (1970) reported that sodium dodecyl sulfate (SDS) activates latent enzymes by solubilizing them. We observed a higher level of latent enzymes solubilized in I^- with respect to I^+ when we added SDS immediately after the irradiation. Hence, it is possible that a high-dose rate (I^+) generates a severe unfolding of the enzyme. As a consequence, PPO presents the hydrophobic groups to the aqueous phase, which repels these groups. As a result, the enzyme solubility is decreased. This assumption is supported by a previous work (Khan and Ali, 1985), in which the authors observed that unfolding of the enzyme was responsible for the photoinactivation of PPO.

The tyrosine hydroxylase activity that requires the subunits HL_2 (74 kDa) and is involved in both monophenolase and diphenolase activities (Kempner and Miller, 1989). It transforms a monophenol into a diphenol, prior to its oxidation. Figures 10 and 11 clearly illustrate that tyrosine hydroxylase substrate is less active than dopa subtsrate. Ingerbrigtsen et al. (1989) and Moore and Flurkey (1989) also noticed such a lower activity of the tyrosine hydroxylase substrate. The lower activity of tyrosine hydroxylase substrate could be related to its need of molecular oxygen. This assumption is based upon experiments carried out by Boekelheide et al. (1979), who compared the hydroxylation reaction of γ-L-glutaminyl-4-hydroxybenzene to the oxidation of γ-L-glutaminyl-3,4-hydroxybenzene by mushroom PPO. Furthermore, Figures 10 and 11 show that tyrosine hydroxylase is more irradiation sensitive than the dopa oxidase. This higher radiosensitivity of PPO tyrosine hydroxylase activity is attributed to the bigger size of the target (Kempner and Miller, 1989). As previously mentioned, tyrosine hydroxylase requires the subunits HL_2 (74 kDa), whereas the dopa oxidase requires only the subunit H (55 kDa) (Kempner and Miller, 1989). Hence, a bigger target (tyrosine hydroxylase) has a higher probability of being bombarded directly by γ rays than a smaller one (dopa oxidase). Therefore, this reduced tyrosine hydroxylase activity would explain, in part, the lower rate of browning of I^-.

As PPO is necessary to initiate phenol oxidation into dark brown melanin (Skou et al., 1974), it is possible

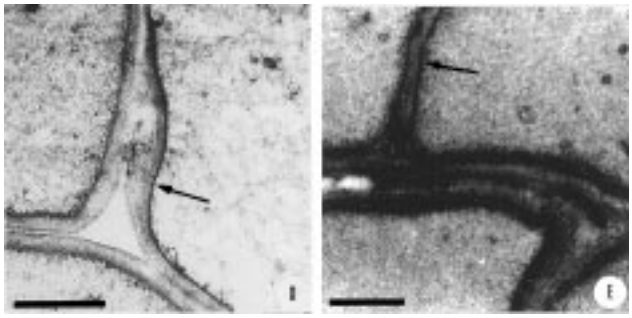


Figure 12. Dose rate effect of irradiation upon mushroom cap cell.

that a decrease of PPO activity would lead to an increase of phenol concentration (GHB) and, hence, to a lower rate of melanin formation. As a matter of fact, mushrooms in I^- demonstrated a preserved coloration of whiteness with a low GHB precursor fluctuation throughout the storage period. However, the PPO activity in I^+ is lower than in I^- during the first 2 days of storage. Likewise, the coloration and browning in I^+ showed a high alteration similar to the control. Therefore, the enzymatic activity cannot be used to rationalize the faster browning in I^+. Because PPO is linked to mitochondria cellular membranes (Vámos-Vigyázó, 1981), we assumed that the cellular membrane was more affected by a high-dose rate (I^+), lowering thus the efficiency of the enzyme (PPO). We observed via electron microscopy that membranes in I^+ are much thicker than in I^- (Figure 12). The thickening of the membranes was also reported by Keresztes et al. (1986). This thickening of the membranes causes a stress to the cell and alters the cell permeability. This alteration allows the release of vacuolar phenolic compounds and the entry of molecular oxygen into the cell cytoplasm. It creates a synergistic effect of enzymatic (PPO) and nonenzymatic (O_2) phenol oxidation. It is also possible that the substrate of other enzymatic pathways is released in the cytoplasm, providing more molecular oxygen upon their reactions. This assumption would explain the browning effect and the smaller concentration of total phenolic compounds observed in I^+. The GHB that should have accumulated in I^+, as it probably did in I^-, upon PPO radioinactivation, was likely oxidized either by enzymatic or by nonenzymatic reactions.

CONCLUSION

This investigation has clearly demonstrated that the dose rate of γ irradiation has a significant effect on the PPO activity, on the browning, and, therefore, on the shelf life of mushrooms *A. bisporus*. A 2 kGy dose of γ radiation was found to control the aging process rate of mushrooms. On the basis of the coloration experiments, mushroom shelf life was extended by 4 days with the lower dose rate irradiation, 4.5 kGy/h, and only by 2 days with the higher dose rate, 32 kGy/h. The enhancement of the shelf life at 4.5 kGy/h was explained by the lower PPO activity that led to an increase of phenol concentration, as confirmed by a low GHB precursor fluctuation throughout the storage and, hence, to a lower rate of melanin formation. However, this explanation does not match the findings at 32 kGy/h, namely, a lower PPO activity and a more important browning effect via melanin formation. The loss of the cellular membrane integrity found in mushrooms treated at 32 kGy/h, and similar to control mushrooms, would alter

the membrane permeability. This would favor both nonenzymatic and enzymatic oxidation of phenols and, thus, would be responsible for the more important browning at 32 kGy/h.

Finally, although mushroom shelf life was improved via 2 kGy treatment at a dose rate of 4.5 kGy/h, work is still required to optimize mushroom preservation. For instance, it would be appropriate to combine low-dose rate irradiations with gas permeable films. Such work is being currently undertaken in our laboratories.

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