Effect of γ-Irradiation on Phenylalanine Ammonia-lyase Activity, Total Phenolic Content, and Respiration of Mushrooms (*Agaricus bisporus*)

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Ionizing treatments were applied at 0.5 kGy, 1.5 kGy, and 2.5 kGy to edible mature mushrooms (*Agaricus bisporus*, *albidus*) in order to assess the effect of the γ-irradiation on some biochemical parameters. Irradiation at doses of 1.5 kGy and 2.5 kGy reduced significantly ($p \leq 0.05$) the rate of respiration of the mushrooms, compared to that of samples irradiated at 0.5 kGy and nonirradiated control samples (**C**). Ionizing treatments increased significantly ($p \leq 0.05$) the phenylalanine ammonia-lyase (PAL) activity and total phenols concentration between days 1 and 4. From days 3−4, to the end of the storage period (day 12), both PAL activity and total phenols in the irradiated samples (**I**) collapsed to lower values. In contrast, the activity of polyphenol oxidase (PPO) increased until days 7, 9, and 12 for samples treated at 0.5, 1, and 2 kGy, respectively. Color measurements showed a loss of whiteness (L* value) during storage. After day 4, however, the effectiveness of γ-irradiation became apparent, and highest L* values were obtained for **I** only.

Keywords: *Agaricus bisporus; phenylalanine ammonia-lyase; phenols; respiration; γ-irradiation*

INTRODUCTION

Agaricus bisporus mushrooms are highly perishable vegetables. Indeed, they can be kept only a few days at 10 °C (Tomkins, 1966) given to browning (Mac Canna and Gormley, 1968), molds (*Verticillium maltousei*) (Salunkle and Desai, 1984), and bacterial contamination (*Pseudomonas tolaasi*) (Royse and Wuest, 1980). To increase their shelf life, there are a number of processes that can be used, such as low-temperature storage (Kovács and Vas, 1974b), controlled-atmosphere packaging (Saxena and Rai, 1988), and chemical treatments (Salunkle and Desai, 1984).

Many researchers have reported low-dose γ-irradiation as a potential tool to extend the postharvest life of many fresh fruits and vegetables (Skou et al., 1974; Thomas, 1988; Lescano, 1994; Gautam et al., 1998; Beaulieu et al., 1992, 1999). However, irradiation with dosages to accomplish the intended purposes has resulted in softening and browning of many fruits and vegetables (Kovács and Vas, 1974a). The development of browning pigments has been associated with several parameters, such as changes in some enzymatic activities, like polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and peroxidase (POD), and in total phenolic compounds level (Long and Alben, 1969; Stüssi and Rast, 1981), as well as the alteration of cellular membrane permeability (Beaulieu et al., 1999).

Phenylalanine ammonia-lyase (PAL) is the key enzyme for the metabolism of phenols (Camm and Towers, 1973). It catalyzes the deamination of L-phenylalanine to yield ammonia and trans-cinnamic acid (Scheme 1), from which phenolic compounds will be produced (Camm and Towers, 1973). Moreover, PAL activity was found to vary greatly with the stage of plant development (Hahlbrock and Scheel, 1989). Various stresses, such as irradiation, wounding, nutrient deficiencies, herbicide treatment, and viral, fungal, and insect attacks, were reported to increase either PAL synthesis or activity in a variety of plants (Chalker-Scott and Fuchigami, 1989; Camm and Towers, 1973).

The effect of the γ-irradiation on the PAL activity in correlation with the content of phenols in edible mushrooms is not yet fully understood. We have, therefore, undertaken an in-depth investigation of the effect of γ-irradiation on some biochemical processes in *Agaricus bisporus* mushrooms. The purpose of this work was to determine the effect of low-dose γ-irradiation on the browning of edible mushrooms. Several biochemical parameters in mushrooms were also assessed during storage, including respiration rate, phenolic content, color, PAL activity, and PPO activity.

MATERIALS AND METHODS

Samples. Forty boxes (22.73 kg each) of edible mature mushrooms (*Agaricus bisporus*, *albidus*) of the *albidus* variety, from Excel producer (Montreal, PQ, Canada) were used for this investigation.

Irradiation Treatments. For the assessment of total phenols, respiration, and PAL, mushrooms were irradiated (Canadian Irradiation Center, Laval, PQ, Canada) in triplicate at doses of 0.5 kGy, 1.5 kGy, and 2.5 kGy with a ^{60}Co source carrier type industrial irradiator (MDS-Nordion International Inc., Kanata, ON, Canada). The irradiation exposure at the ^{60}Co source varied with the applied dose. PPO activity and the color of the mushrooms were investigated with a similar system, but at a later time. Because the cobalt source loses 10% of its strength per year, lower doses were applied to assess the effect of the irradiation on the latter parameters, at 0.5 kGy, 1.0 kGy, and 2.0 kGy.

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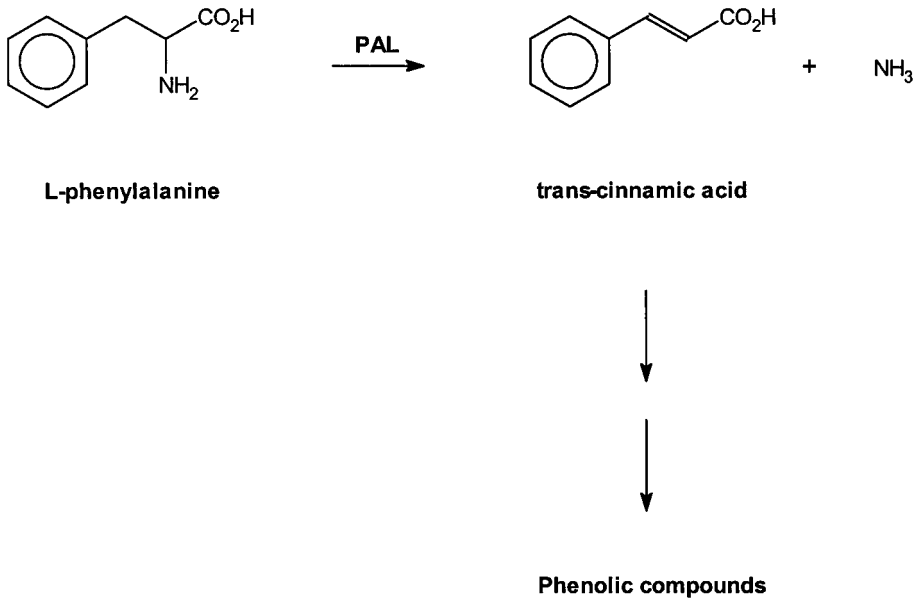
Scheme 1



Storage Conditions. Treated (**I**) and control (**C**) mushrooms were stored at 4 °C and 72% RH in separate, open containers.

Analyses. Mushroom analyses began on day 0 and were performed daily until days 8−9 for both **C** and **I** samples. For the assessment of PPO activity and the whiteness, the storage lasted 11 days.

Respiration. For each treatment, mushrooms (200 g) were removed from open containers and placed in a desiccator. Respiration was studied at a constant temperature of 18 °C by absorption of carbon dioxide evolved from the mushrooms during 120 min each day and for 8 days of storage. An air sample (50 cm^3) of the headspace was taken up and the level of CO_2 was determined with a GC system (Varian 3400 GC) equipped with a flame ionization detector, Haysep N column (2 m × 80−100 mesh), combined with a molecular sieve column (2 m × 45−60 mesh). The injector and detector temperatures were 60 °C and 120 °C, respectively. The temperature of the oven was 80 °C. The carrier gas was helium and the flow rate for molecular sieve column was 30 mL/min. The results are expressed in mL/kg/min.

Total Phenolic Content. The total phenolic compounds were extracted using a modification of the procedure described by Coseteng and Lee (1987). Eighty grams of mushrooms were homogenized in a Waring blender with 100 mL of 80% ethanol for 2 min. The homogenate was boiled for 5 min under the hood. The extract was filtered through Whatman #4 filter paper. The residue was mixed with an additional 100 mL of 80% ethanol and boiled for 10 min to re-extract the phenolic compounds. The extracts were combined and the solution was allowed to cool before it was made up to a final volume of 250 mL. This extract was used for the determination of total phenolic content.

Total phenolic content was determined by the procedure described by Weurman and Swain (1955). One mL of the alcohol extract was added to 10 mL of distilled water. Two mL of Folin-Ciocalteu phenol reagent (Sigma Chemical Company, St. Louis, MO) was added. The sample was mixed, and after 5 min 2 mL of saturated sodium carbonate solution was added and then the mixture was shaken. The optical density of the solution was measured at 640 nm after 1 h using a DMS 200 Spectrophotometer (Varian Canada Inc., Pointe-Claire, PQ, Canada). The total phenolic content was calculated from a standard curve of chlorogenic acid (10−320 μg/mL) prepared at the same time.

Color Measurements. The measurements of mushroom whiteness (L*) were performed with a Colormet colorimeter (Instrumar Ltd, St. John, NF, Canada) on fifteen mushrooms per sample. This apparatus measures the light reflection spectrum and converts it into L*, a*, and b* Cartesian coordinates of a three-dimensional space map of all colors (CIELAB, 1976).

PAL Activity. The extraction and activity of PAL were determined using a modification of the procedure described by Tan and Lam (1985). Eight grams of frozen mushrooms were ground in liquid nitrogen to a fine powder. Two hundred mg of poly(vinylpyrrolidone) (PVP) (Sigma Chemical Company, St. Louis, MO) was added, followed by 9 mL of 0.1 M tetraborate buffer, pH 8.7. The homogenate was centrifuged (20 000*g* for 30 min) and then incubated at 37 °C for 2 h. The reaction was terminated by the addition of 40 μL of 6M HCl. The optical density was measured at 280 nm, with a DMS 200 Spectrophotometer (Varian Canada Inc., Pointe-Claire, Canada). The PAL activity was calculated from a standard curve of trans-cinnamic acid (10−90 μg/mL) prepared at the same time.

Polyphenol Oxidase Activity. The extractions of polyphenol oxidase (PPO) were performed with acetone powder (Frylink et al., 1987). Poly(ethylene glycol) 4000 (1.5 g) and acetone (45 mL, at −20 °C) were added to 7.5 g of mushrooms. The mixture was homogenized for 1 min. The homogenate was filtered with a sintered glass filter (G3). The acetonic powder was homogenized with 15 mL of 10 mM buffer acetate (4 °C) pH 5, and centrifuged (10 000*g*, 30 min). The precipitate was discarded, and two volumes of acetone were added to the supernatant and centrifuged (20 000*g*, 30 min). The supernatant was discarded and the precipitate was re-suspended in 7.5 mL of 10 mM buffer acetate pH 5.

To determine the PPO activity, 1.0 mL of the above solution was added to 4 mL of a solution constituting 10 mM buffer acetate pH 5 (10 mL), $CuSO_4$ 0.13% (0.1 mL), and catechol oxidase (0.02 g in 2.0 mL of 10 mM buffer acetate pH 5) (Sigma Chemical, St. Louis, MO). The absorbance was immediately measured at 30 °C with a Varian DMS 200 spectrophotometer at 480 nm every 15 s over a period of 10 min. The PPO activity is expressed in terms of $\Delta A_{480\ nm}$/min.

Statistical Analyses. Results were submitted to an analysis of variance followed by Duncan multiple-range tests. The homogeneity of variances was assessed by Bartlett's test. The degree of confidence was fixed at 95% for all the analyses (Snedecor and Cochran, 1978). For each measurement, three replicates of five mushrooms were tested.

RESULTS

Respiration. The respiration results of the nonirradiated (**C**) and irradiated (**I**) mushrooms during storage are presented in Figure 1. The production of CO_2 in **C**

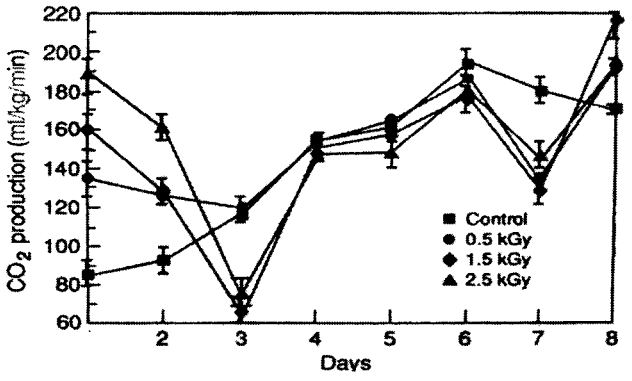


Figure 1. Mushroom respiration rate during the storage period (production of CO_2 mL/kg/min).

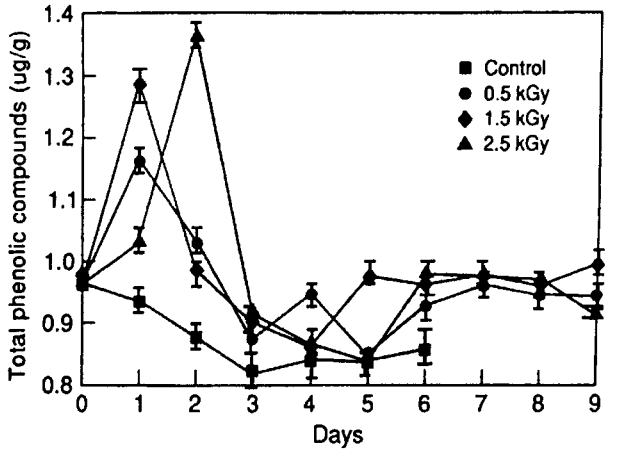


Figure 2. Total phenols content in mushroom during the storage period (μg of chlorogenic acid/g fresh weight).

increased significantly ($p \leq 0.05$) to reach a maximum value at day 6. Upon irradiation treatment, a significant ($p \leq 0.05$) increase of CO_2 production was observed during the first 24 h, followed by a significant decrease ($p \leq 0.05$) between days 1 and 3. The respiration of the **I** mushrooms was at its lowest value on day 3. However, from days 3−8, the mushrooms respiration increased significantly ($p \leq 0.05$).

Total Phenols Content. The variation of total phenols content in **C** and **I** during storage is represented in Figure 2. Total phenols content of **C** decreased significantly ($p \leq 0.05$) during the storage to reach a minimum at day 3 and remained stable until day 6. Gamma irradiation resulted in a significant ($p \leq 0.05$) increase of the total phenols content. The highest values were obtained at day 1 in mushrooms irradiated at 0.5 kGy and 1.5 kGy and at day 2 in mushrooms irradiated at 2.5 kGy (Figure 2). The total phenols content was directly linked to the dose of irradiation applied, viz. 1.17, 1.28, and 1.38 μg/g for mushrooms treated at 0.5, 1.5, and 2.5 kGy, respectively. After day 3, total phenols content decreased significantly ($p \leq 0.05$) in **I**, but remained significantly ($p \leq 0.05$) higher than that in **C** mushrooms (Figure 2).

PAL Activity. The effect of γ-irradiation on the PAL activity is presented in Figure 3. Irradiation treatment increased significantly ($p \leq 0.05$) the PAL activity of the mushrooms when compared to that of **C**. Highest PAL activity ($p \leq 0.05$) was observed at day 2 for samples treated at 0.5, 1.5, and 2.5 kGy. At day 2 of storage, PAL activity was 4-fold higher in samples treated at 2.5 kGy than in **C**, and 2.5-fold higher in

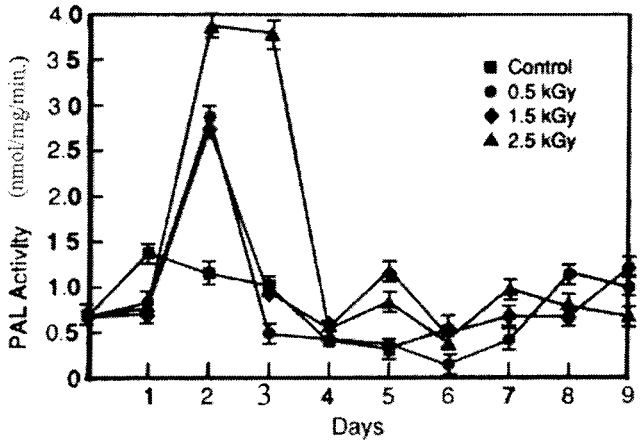


Figure 3. Mushroom PAL activity during the storage period (nmol/mg/min).

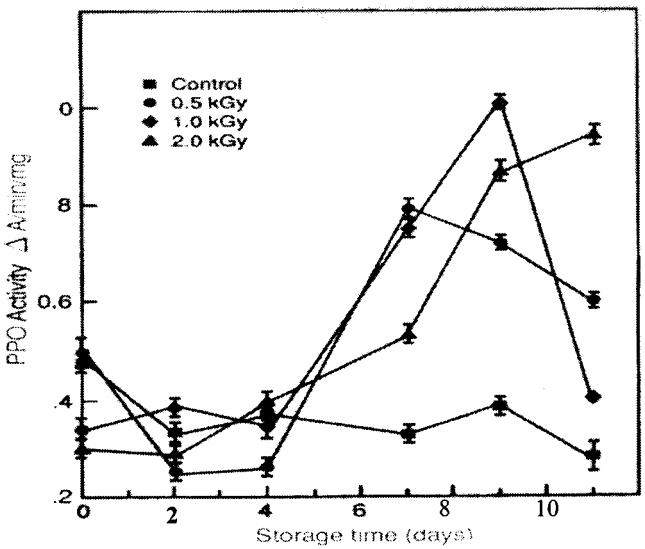


Figure 4. Mushroom PPO activity during the storage period (ΔA/min/mg, where ΔA represents change in absorbance).

samples treated at 0.5 or 1.5 kGy than in **C**. After day 2 for samples irradiated at 0.5 and 1.5 kGy, and after day 3 for samples irradiated at 2.5 kGy, PAL activity decreased significantly ($p \leq 0.05$). As for the control mushrooms, a slight increase of PAL activity appeared at day 1, followed by a weak decrease until the end of the experiment. Until day 4, the PAL activity in **C** was clearly lower ($p \leq 0.05$) than in **I**.

Polyphenol Oxidase Activity. The polyphenol oxidase (PPO) activity was assessed by its catechol oxidase activity (Figure 4). Immediately after the irradiation treatment, mushrooms irradiated at 1.0 and 2.0 kGy exhibited a significantly ($p \leq 0.05$) lower PPO activity, when compared to nonirradiated controls (**C**) and mushrooms irradiated at 0.5 kGy. During the storage, a significant increase ($p \leq 0.05$) of the activity was observed. A maximum of PPO activity appeared at days 7 and 9 for samples treated at 0.5 and 1 kGy, respectively, and was followed by a decrease. For mushrooms irradiated at 2.0 kGy, PPO activity continued to increase until the end of the experiment (9 days). No significant fluctuation of the catechol oxidase activity in **C** was observed during the storage.

Color Measurements. During storage, a decrease of whiteness was observed for all mushrooms, both control and treated (Figure 5). Between the beginning

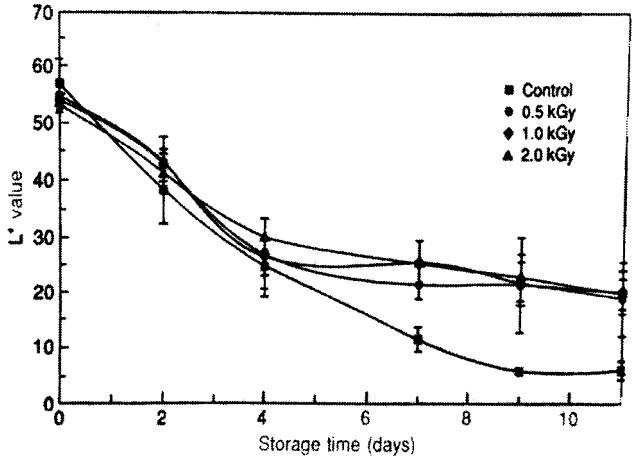


Figure 5. Mushroom browning during the storage period.

of the storage and day 4, both **C** and **I** samples behaved similarly, viz. L* values appeared to decrease significantly ($p \leq 0.05$), indicating that browning of the mushrooms was taking place during the storage. However, from day 4 until the end of the experiment at day 11, L* values of **I** remained almost constant, while L* values in **C** samples continued to decrease. At day 11, all irradiated mushrooms exhibited a L* value of about 20, independent of the dose applied, compared to a value of 5 for **C**.

DISCUSSION

Gamma-irradiation caused a significant ($p \leq 0.05$) enhancement of the respiration rate of the mushrooms after 24 h. This behavior might be explained by the stress coming from the irradiation treatment. The stress caused by the irradiation treatment increases progressively according to the dose of irradiation. A similar behavior was previously observed by Ajlouni et al., 1993 in irradiated mushrooms. The level of CO_2 produced by the mushrooms was 135, 160, and 190 mL/kg/min for samples treated at 0.5, 1.5, and 2.5 kGy respectively, as compared to 85 mL/kg/min for **C**. Therefore, an increase of the metabolism of the mushrooms was expected during the first 24 hours following the irradiation. However, the respiration rate profile in **I** is different from that in **C**. After 24 hours, the production of CO_2 fell to lower levels for mushrooms irradiated at 1.5 kGy and 2.5 kGy, whereas an increase of the respiration was observed in **C** and mushrooms irradiated at 0.5 kGy. A fall of CO_2 production within a few days after the irradiation treatment was also observed by Ajlouni et al., 1993 for mushrooms treated at 1 kGy and by Kovács and Vas (1974b) for mushrooms irradiated at 3 kGy. From day 3 to the end of the storage, an increase of the respiration rated occurred subsequently for **I** and **C** samples. However, mushrooms irradiated at 1.5 and 2.5 kGy had a substantially smaller respiration rate than **C** and the 0.5 kGy irradiated samples. A lower respiration rate is linked to a reduction of the metabolic activity. Ajlouni et al., 1993 observed a climateric-like increase in the rate of respiration of unirradiated mushrooms during the first 5 days of storage, as compared to that of irradiated mushrooms.

Gamma-irradiation of mushrooms caused an enhancement of the PAL activity in the early stages of the storage period, which thereafter decreased on days 3−4 to constant values. However, these values remained higher in mushrooms irradiated at 1.5 and 2.5 kGy than the values of control mushrooms and mushrooms irradiated at 0.5 kGy. Such enhancement of the PAL activity is in agreement with previous reports on irradiated citrus (Riov et al., 1968), grapes (Riov et al., 1970), apples (Tan, 1980), clementines (Oufedjikh et al., 2000), and potatoes (Pendharkar and Nair, 1977). As PAL is involved in the synthesis of some phenols in plants and fruits (Camm and Towers, 1973), its enhancement during the first days of storage in irradiated mushrooms is accompanied by an increase of total phenols during the same period of time (Figure 2). The accumulation of phenols in irradiated mushrooms is an important factor in microorganisms resistance, and thus the shelf life, because phenols could contribute to antimicrobial properties (Tan and Lam, 1985). Thereafter, the total phenols content in **I** decreased, but was still higher than in **C**, which behavior is in direct relation to the reduction of the PAL activity at the end of the storage.

During the first 24 hours, PPO activity in 1 and 2 kGy samples was lower than that in 0.5 kGy and **C** mushrooms. Such behavior is in agreement with results reported earlier (Gautam et al., 1998; Beaulieu et al., 1999). 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. As PPO is necessary to initiate phenols oxidation into dark-brown melanin (Skou et al., 1974), it is possible that the observed increase of PPO activity at the end of the storage period might also explain the decrease of phenols concentration during the same period of time. As a matter of fact, the maximum of PPO activity arose between days 7 and 9, which is the period of the lowest level of total phenols and PAL activity (end of the storage), whereas the maximum of total phenols appeared between days 1and 3, which corresponds to the period of the lowest level of PPO activity (early in the storage). Furthermore, color measurements revealed a decrease of the L* value upon storage, confirming the loss of whiteness of the mushrooms due, most probably, to the formation of melanin (Beaulieu et al., 1999). However, after day 4, L* values in irradiated samples were significantly higher ($p \leq 0.05\%$) than those in control samples. These results confirmed the beneficial effect of γ-irradiation to delay the browning process of mushrooms during storage and thus, to preserve the whiteness for a longer period of time when compared to nonirradiated control mushrooms. (Beaulieu et al., 1999).

CONCLUSION

This investigation has clearly evidenced that γ-irradiation of *Agaricus bisporus* mushrooms activates some biochemical parameters, such as PAL activity, total phenols content, PPO activity, respiration, and whiteness. Gamma-irradiation caused a significant increase of PAL activity of *Agaricus bisporus* mushrooms at the early stage of storage (days 1−4). As PAL is directly linked to the synthesis of phenols, γ-irradiation provoked also a significant increase of total phenols in *Agaricus bisporus* mushrooms (days 1−3). Gamma-irradiation reduced the rate of respiration of *Agaricus bisporus* mushrooms. The reduction of respiration intensity was accompanied by the delay of the browning, as confirmed by the coloration experiments.

Finally, a dose of 1.5 kGy and 2.5 kGy seemed to be the treatment to optimize mushroom preservation. For instance, it would be appropriate to combine both low dose irradiations to gas-permeable films. Such work is being currently undertaken in our laboratories.

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