

Full Length Research Paper

## Gamma irradiation can control the number of psychotrophic bacteria in *Agaricus bisporus* during storage

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We evaluated the effect of gamma irradiation doses (0, 125, 250, and 500 Gy) in control of psychotrophic bacteria in different strains of *Agaricus bisporus* (ABI-07/06, ABI-05/03, and PB-1) during storage, cultivated in composts based on oat straw (*Avena sativa*) and *Brachiaria* spp. The experimental design was completely randomized in a factorial scheme 4 × 2 × 3 (irradiation doses × composts × strains), with 24 treatments, each consisting of 2 replicates, totaling 48 experimental units (samples of mushrooms). The mushrooms collected from all culture conditions were packaged in plastic polypropylene with 200 g each and subjected to Cobalt-60 irradiator, type Gammacell 220, and dose rate 0.740 kGy h<sup>-1</sup>, according to the treatments. Subsequently, the control (nonirradiated) and other treatments were maintained at 4 ± 1°C and 90% relative humidity (RH) in a climatic chamber to perform the microbiological analysis of mushrooms on the 1st and 14th day of storage. According to the results, it was found that the highest mean colony psychotrophic count, after 14 days of storage, was observed in strain ABI-07/06 [1.30 × 10<sup>8</sup> g<sup>-1</sup> most probable number (MPN)] in nonirradiated mushrooms, coming from *Brachiaria* grass-based compost, and this same strain under the same storage conditions, coming from the same type of compost that underwent a dose of 500 Gy, obtained a significant reduction in mean colonies of psychotrophic bacteria (2.25 × 10<sup>4</sup> g<sup>-1</sup> MPN). Thus, the irradiation doses tested favored reducing the number of colonies of psychotrophic bacteria, regardless of the type of compound and strain of *A. bisporus*.

**Key words:** Champignon, shelf life, postharvest, mushrooms.

### INTRODUCTION

Edible mushrooms are a food of high nutritional quality. However, the useful life of the mushrooms as well as

their nutritional value vary depending on the species, strain, processing postharvest, developmental stage of

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mushroom, and the type of substrate used (Andrade et al., 2008; Bononi et al., 1995; Minhoni et al., 2005). As mushrooms are highly perishable, they tend to lose the quality immediately after harvest. The short shelf life (1 to 3 days at room temperature) is a disadvantage for the distribution and marketing of fresh product. In this period, changes occur in the mushroom, such as darkening, opening of the pileus, stem elongation, increase in the diameter of the hat, weight loss, and change in texture due to high respiration rate and lack of physical protection to prevent water loss or microbial attack (Akram and Kwon, 2010; Sommer et al., 2010; Singh et al., 2010).

Food irradiation is one of the best and most satisfactory techniques for food preservation. However, the irradiation dose required to control microorganisms on food depends on various factors such as the strength of each particular kind and degree of contamination of the food. For edible mushrooms, irradiation commonly is performed in many countries with low doses (1 to 3 kGy), aiming to reduce the number of spoilage microorganisms and prolonging its life and sensory qualities (Fernandes et al., 2012; Farkas, 2006).

In Brazil, it is still common practice to irradiate mushrooms as a method for postharvest storage, and for *Agaricus bisporus*, the production is largely marketed in the preservative solution, for instance, calcium chloride and glucanallactone (Kuyper et al., 1993; Rodrigo et al., 1999). However, it is known that such solutions change the original taste of fresh mushrooms and their physicochemical and sensory properties. Recently, Moda (2008) evaluated the shelf life of *Pleurotus sajor-caju* irradiated with 125, 250, 500, and 750 Gy and found that a dose of 750 Gy was the most suitable and its estimated shelf life of five days, being this time superior for nonirradiated mushrooms. However, in Brazil, no scientific reports were published of the use of gamma radiation to *A. bisporus*, being necessary to check the feasibility of applying this technique in growing conditions prevailing in the country. Thus, we evaluated the effect of irradiation doses (0, 125, 250, and 500 Gy) in control of psychrotrophic bacteria in strains of *A. bisporus* (ABI-07/06, ABI-05/03, and PB-1) grown in two kinds of composts (brachiaria and oats) during 14 days of storage at  $4 \pm 1^\circ\text{C}$  and 90% RH.

## MATERIALS AND METHODS

The production of mushrooms was developed on the premises Mushroom Module, Department of Plant Protection, Faculty of Agronomic Sciences (FCA/UNESP), Botucatu/SP, Brazil.

### Experimental design and treatments

The experimental design was a factorial  $4 \times 3 \times 2$  (irradiation doses  $\times$  strains  $\times$  composts), with 24 treatments, each consisting of 2 replicates, totaling 48 experimental units (samples of mushrooms).

Data were subjected to analysis of variance, and means were compared by Tukey test, using the SISVAR 4.2 statistical program, developed by Department of Mathematical Sciences from UFLA (Federal University of Lavras), MG, Brazil.

### Strains of *Agaricus bisporus*

We used pure culture (primary matrix) strains ABI-07/06, ABI-05/03, and PB-1; the strain ABI-07/06 originated from Piedade/SP, the strain ABI-05/03 originated from Cabreúva/SP, and the strain PB-1 from the company Brasmicel (Suzano-SP), and that, according to the same log files, was acquired by Paul Stamets (Washington, USA) in 2000. These strains are maintained in culture stock, the base culture medium (CA compost agar) submerged in mineral oil sterilized and maintained in biological oxygen demand (BOD) adjusted to  $8^\circ\text{C}$ , the Bank Matrix Module mushrooms, located at the Department of Production Plant of the Faculty of Agricultural Sciences, UNESP, Botucatu/SP, Brazil.

### Composting, growing, and harvesting

We formulated two types of composts based on brachiaria (*Brachiaria* spp.) and oat (*Avena sativa*) straws, with an initial C/N ratio of about 25/1, for the cultivation of *A. bisporus* strains. All stages of composting, growing, and harvesting followed procedures used by Andrade et al. (2008).

### Postharvest

The mushrooms collected from all culture conditions proposed were packaged in plastic polypropylene boxes each containing 200 g. Transport of samples was done in cool boxes to the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Brazil, where they were irradiated with 125, 250, and 500 Gy in Cobalt-60 irradiator, type Gammacell with 220 kGy dose rate  $0.740 \text{ h}^{-1}$ . The control (nonirradiated) and other treatments were maintained at  $4 \pm 1^\circ\text{C}$  and 90% of moisture in an incubator for the realization of physicochemical analyzes on the 1st and 14th day of storage.

### Sample preparation and dilutions

Analyses were performed on samples of 25 g of fresh mushrooms, weighed aseptically and placed in sterile elemeyers with 225 ml of peptone water (0.1%) sterile, constituting  $10^{-1}$  dilution after stirring for 2 min in a peristaltic homogenizer. One milliliter of  $10^{-1}$  dilution was pipetted in 9 ml of sterile peptone water (0.1%) and from this dilution  $10^{-2}$ ,  $10^{-3}$  to  $10^{-6}$  dilutions were tested.

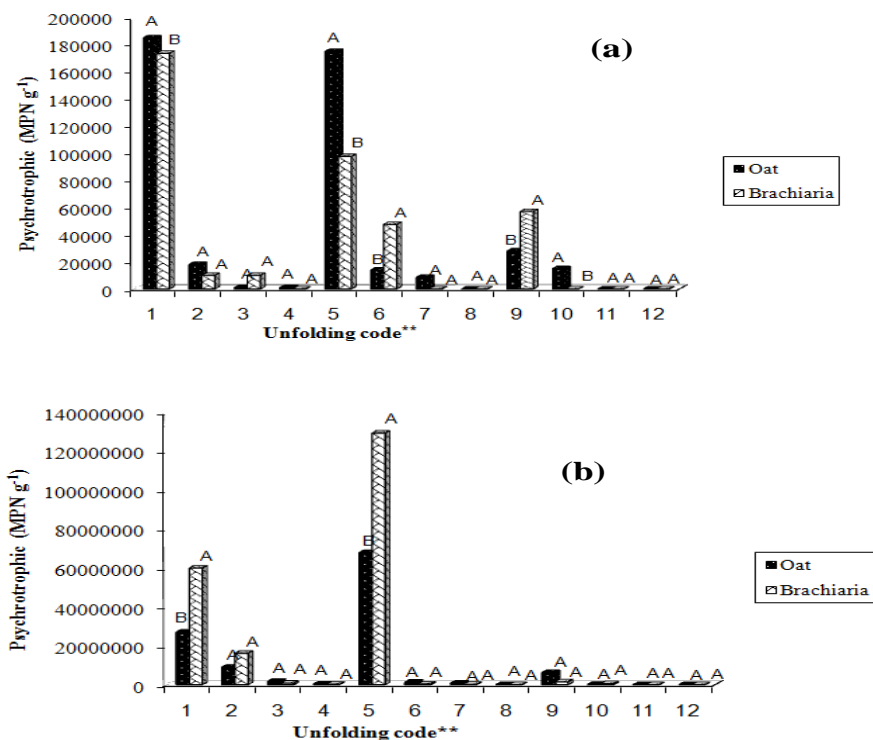
### Psychrotrophic count

We used for the enumeration of psychrotrophic bacteria the dilutions  $10^{-3}$  to  $10^{-6}$  in duplicates. We added 0.1 ml of dilution in Petri dishes containing culture medium plate count agar (PCA) previously prepared and sterilized, these being subsequently inverted and incubated at  $7^\circ\text{C}$  for 10 days. After this period, we counted the number of colonies by MPN  $\text{g}^{-1}$  food (most probable number). This same methodology procedure and evaluation was repeated after 14 days of storage of the mushroom samples. Thus, microbiological evaluations were made for two periods (on the 1st and 14th day of storage), totaling 384 Petri dishes, with 192 in each period.

**Table 1.** F values from analysis of variance of the number of psychrotrophic microorganisms colonies present in mushrooms of *Agaricus bisporus* strains ABI-05/03, ABI-07/06, and PB-1, newly irradiated (initial), and stored at  $4 \pm 1^\circ\text{C}$  for 14 days (final), grown on two kinds of composts based on oat straw and *Brachiaria* and submitted to irradiation doses 0, 125, 250, and 500 Gy.

Variation cause	Psychrotrophic (initial)	Psychrotrophic (final)
Strain (S)	236.75**	90.66**
Compost (C)	7.84**	29.25**
Dose irradiation (D)	1450.94**	261.72**
S x C	10.59**	10.58**
S x D	173.98**	91.43**
C x D	12.90**	25.62**
S x C x D	42.29**	11.20**
CV (%)	14.7	37.4

\*\* Significant at 1%.

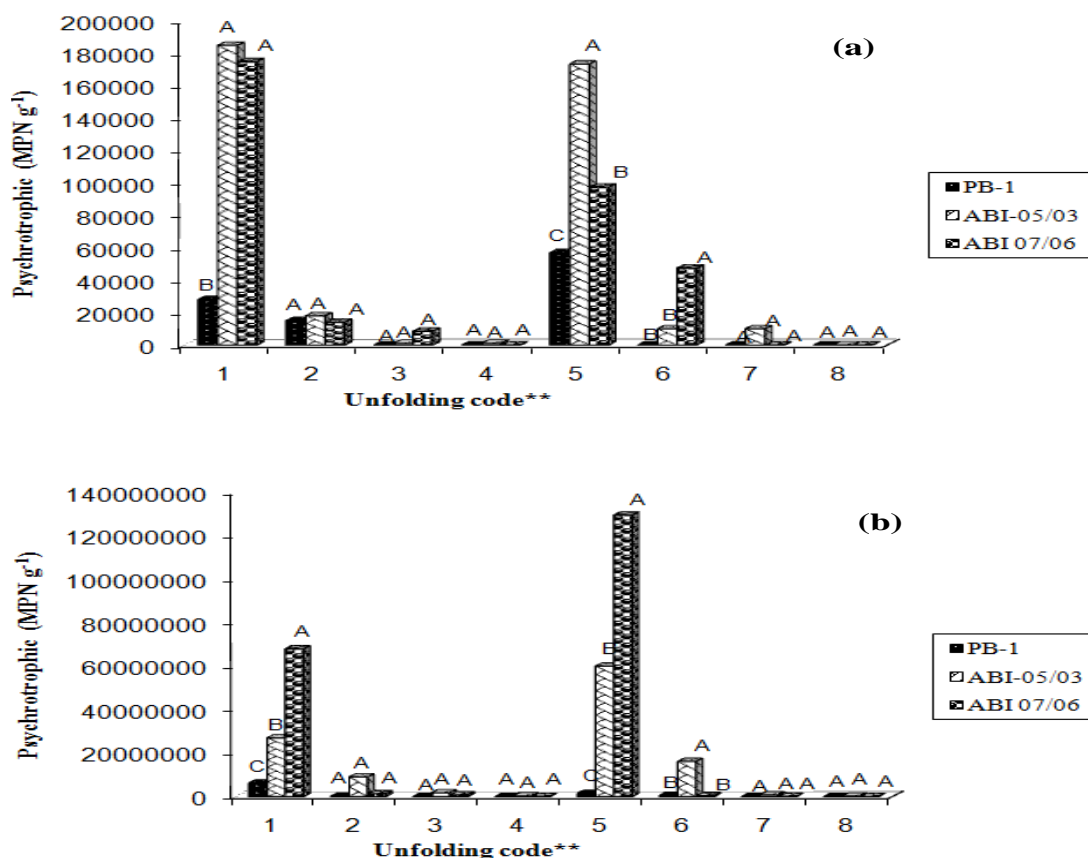


**Figure 1.** Comparison of mean number of psychrotrophic microorganisms colonies in newly irradiated mushrooms (a) and stored at  $4 \pm 1^\circ\text{C}$  for 14 days (b), in function of the type of compost. Means followed by the same letter within each evaluation period and unfolding encoding are not statistically different from each other (Tukey, 5%).\*\* *Agaricus bisporus* Strain - Dose Irradiation: 1- ABI 05/03 - 0, 2- ABI 05/03 - 125, 3- ABI 05/03 - 250, 4- ABI 05/03 - 500, 5- ABI 07/06 - 0, 6- ABI 07/06 - 125, 7- ABI 07/06 - 250, 8- ABI 07/06 - 500, 9- PB-1 - 0, 10- PB-1 - 125, 11- PB-1 - 250, 12- PB-1 - 500.

## RESULTS AND DISCUSSION

F values from analysis of variance of the number of psychrotrophic bacterial colonies present in *A. bisporus* are shown in Table 1. For all strains of *A. bisporus* tested

at doses of 250 and 500 Gy, there was no significant difference in the number of psychrotrophic bacterial colonies from mushrooms grown on composts based on oats and *Brachiaria* grass, both in newly irradiated and in stored mushrooms (Figure 1). Moreover, in nonirradiated



**Figure 2.** Comparison of mean number of psychrotrophic microorganisms colonies in newly irradiated mushrooms (a) and stored at  $4 \pm 1^\circ\text{C}$  for 14 days (b), in function of the strain of *Agaricus bisporus*. Means followed by the same letter within each evaluation period and unfolding encoding are not statistically different from each other (Tukey, 5%).\*\*Compost - Dose Irradiation: 1- Oat - 0, 2- Oat - 125, 3- Oat - 250, 4- Oat - 500, 5- Brachiaria - 0, 6- Brachiaria - 125, 7- Brachiaria - 250, 8- Brachiaria - 500.

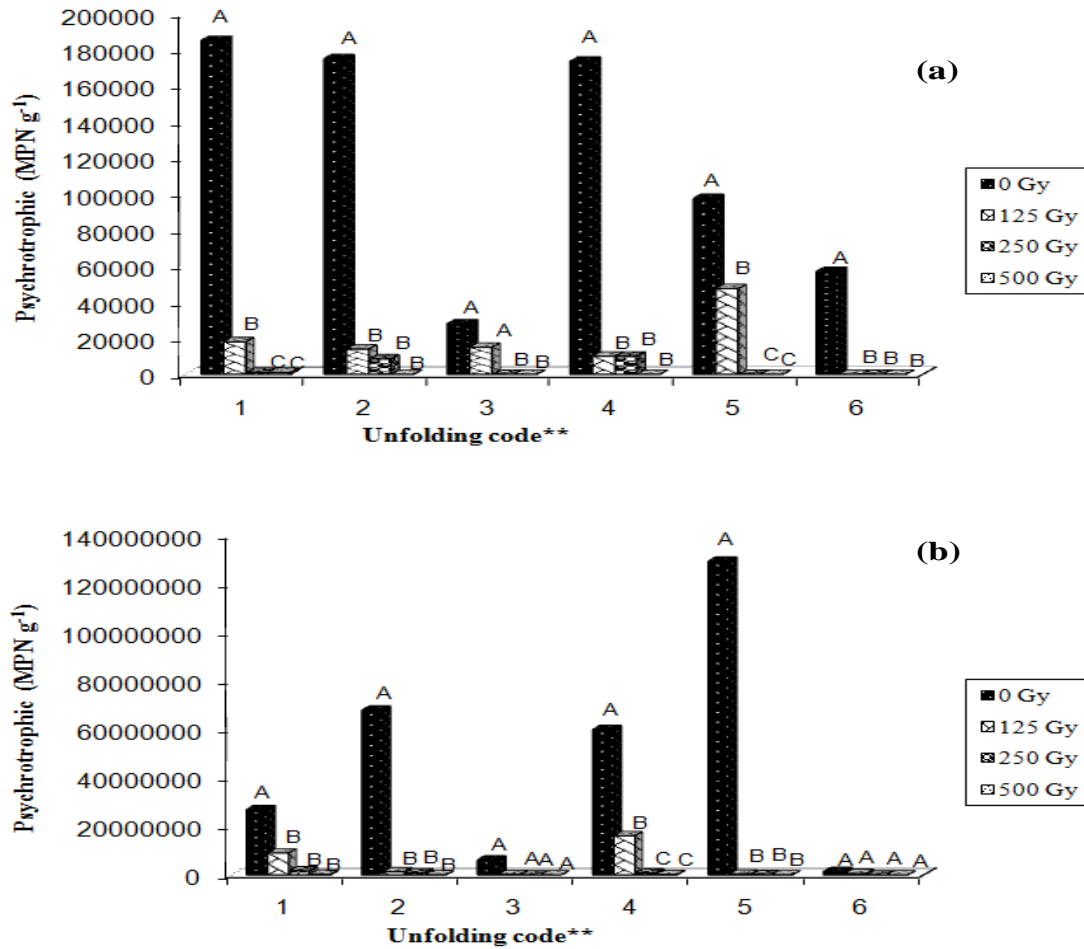
(control) mushrooms, the number of psychrotrophic bacteria was influenced by the compost type. These results indicate that irradiation at doses of 250 and 500 Gy was effective in eliminating the effect of the compost on the number of psychrotrophic bacteria and indicated a significant reduction of microorganisms compared to control in both periods.

The highest average number of psychrotrophic colonies, after 14 days of storage, was observed in the ABI-07/06 strain ( $1.30 \times 10^8$  MPN  $\text{g}^{-1}$ ) in nonirradiated mushrooms, coming from *Brachiaria* compost (Figure 1). This same strain under the same storage conditions, coming from the same compost type but after a dose of 500 Gy, showed a significant reduction in mean number of psychrotrophic bacterial colonies ( $2.25 \times 10^4$  MPN  $\text{g}^{-1}$ ). These results differ from those obtained by Moda (2008), which evaluated the increased shelf life mushroom *P. sajor-caju* with application of gamma radiation and also found that the average psychrotrophic bacteria at a dose of 500 Gy ( $4.5 \times 10^8$  MPN  $\text{g}^{-1}$ ) in mushrooms *P. sajor-caju* stored for 10 days was not lower than the average obtained on samples of nonirradiated mushrooms ( $2.5$

$\times 10^7$  MPN  $\text{g}^{-1}$ ).

It is known that the type of mushroom and irradiation dose applied directly influence the results of increasing the mushrooms' shelf life. About this, Rivera et al. (2011) evaluated the effect of gamma irradiation on the microbial population of *Tuber melanosporum*; during 35 days of storage at  $4^\circ\text{C}$ , they found that the dose of 1500 Gy did not increase the useful life of mushrooms and maintenance of the quality of truffles. Moreover, Jiang et al. (2010), evaluating the effect of integrated application of gamma irradiation (1000, 1500 and 2000 Gy) and modified atmosphere on the microbiological properties of shiitake mushroom (*Lentinula edodes*), found that the dose of 1000 Gy was the most efficient in maintaining the firmness level of mushrooms.

Comparing the mean number of psychrotrophic bacterial colonies of strains of *A. bisporus*, it was found that there were significant differences in the nonirradiated treatments in two periods (Figure 2). Furthermore, in samples of mushrooms subjected to doses of 250 and 500 Gy, the values for the strains of *A. bisporus* were similar to each other as was the reduction in the number



**Figure 3.** Comparison of mean number of psychrotrophic microorganisms colonies in newly irradiated mushrooms (a) and stored at 4 ± 1°C for 14 days (b), in function of irradiation dose. Means followed by the same letter within each evaluation period and unfolding encoding are not statistically different from each other (Tukey, 5%). Compost – *Agaricus bisporus* Strain: 1- Oat – ABI-05/03, 2- Oat – ABI-07/06, 3- Oat – PB-1, 4- Braquiaria - ABI-05/03, 5- Braquiaria – ABI-07/06, 6- Braquiaria – PB-1.

of psychrotrophic bacteria (compared to control) in both irradiated and fresh mushrooms stored, resulting in an increase in the mushrooms' shelf life. Beaulieu et al. (2002) also reported the benefit of preserving irradiation on the mushroom *A. bisporus* reporting that the useful life was extended to 4 days with a dose of 4500 Gy of irradiation. Also, Lescano (1994) reports that the dose of 3000 Gy, combined with Poly-Vinyl Chloride (PVC) film packaging and storage of 10 ± 2°C, increased the shelf life of mushrooms *A. bisporus*, providing a white color retention, and growth and the opening of the pileus, acceptable for commercialization for 11 days and for consumption up to 16 days of storage.

Regarding the mean colonies of psychrotrophic bacterial colonies on mushrooms when submitted to irradiation, it was found that all doses tested favored reducing these colonies compared to control (Figure 3). For ABI-05/03 strain grown on oat-based compost, the average psychrotrophic colony count in the nonirradiated

treatment was 1.85 × 10<sup>5</sup> MPN g<sup>-1</sup>. Already at a dose of 500 Gy, this number was reduced to 1.00 × 10<sup>3</sup> MPN g<sup>-1</sup>. Similar results were obtained by Moda (2008) who also found a reduction of the number of psychrotrophic bacteria in *P. sajor-caju* between 1 day before and 1 day after irradiation (500 Gy) of 6.2 × 10<sup>6</sup> MPN g<sup>-1</sup> for 1.3 × 10<sup>4</sup> MPN g<sup>-1</sup>, respectively.

### Conclusion

The irradiation doses tested favored the reducing number of psychrotrophic bacteria colonies, regardless of composts and *A. bisporus* strains.

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