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# Influence of Selected Cultural Factors on Relative Tyrosinase Activity in Cultivated Mushrooms

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ABSTRACT: Dry compost and addition of copper to the casing layer (2 times normal) was found to modestly increase the relative tyrosinase activity (RTA) isolated from an off-white strain (PSU #211) of *Agaricus bisporus* by a commercial chemical manufacturer. However, the greatest increase in RTA was observed with increasing flush of the crop cycle independent of cultural treatment. Also, RTA was found to correlate (r=0.91) with copper content accumulated in the mushrooms and their tendency for postharvest browning (r=0.88). Data from a second replicate crop yielded similar results. However, in a third crop where the only difference was that additional copper was added to the compost (2 times normal) at spawning the RTA obtained from the entire crop was approximately doubled. The results indicate that alteration in some cultural practices can increase the amount of the enzyme as a natural product that can be obtained from mushrooms and, conversely, suggest means for growers to improve quality and shelf life of their product.

## 1 INTRODUCTION

Tyrosinase (monophenol, dihydroxyphenylalanine: oxygen oxido-reductase, ED 1.14.18.1) is a copper-containing enzyme which catalyses two reactions in which molecular oxygen is the hydrogen acceptor and phenol is the hydrogen donor:

A. monophenol + $O_2$ + $AH_2$ → *o*-dihydroxyphenol + $H_2O$ + A
B. *o*-dihydroxyphenol + 1/2 $O_2$ → *o*-quinone + $H_2O$,

where $AH_2$ represents a hydrogen donor (Mason 1965).

Enzymatic oxidative browning, that occurs so dramatically in mushroom tissue after tissue damage, or senesence, is caused by the oxidation of phenols catalyzed by tyrosinase followed by chemical reactions that convert the oxidative products into the brown polymer known as melanin (Burton *et al.* 1993). Tyrosinase is found widely in nature, but cultivated mushrooms (*Agaricus bisporus*) are generally acknowledged to be the major natural source for tyrosinase as a natural product. Anedoctotal evidence has indicated that certain cultural practices, such as compost moisture and presence of excess copper can result in mushrooms more prone to browning. We speculated that this could possibly indicate increased tyrosinase activity of the mushrooms. Hence, the purpose of this investigation was to determine if certain cultural practices, previously observed to increase the suceptability of mushrooms to browning, actually increase tyrosinase activity. Hypothetically, this knowledge could then be used to improve the yield of enzyme as a natural product and, conversely, by mushroom growers to improve the quality of their crops.

## 2 MATERIALS AND METHODS

All mushroom crops were grown at the Mushroom Test Demonstration Facility (MTDF) using standard practices (Beyer and Beelman 1995) unless otherwise stated. A traditional off-white spawn strain (PSU #211) produced by Alpha Spawn Company (Avondale, PA) was employed. Compost moisture was varied in half of each crop to simulate normal moisture (~72% at spawning) and dry compost (~67% at spawning) by altering the amount of water added during Phase I composting and prior to Phase II composting. In two crops (#2006 and #2017) copper was added to the casing in half the trays in the growing room by the addition of 0.024% $CuSO_4 \bullet 5H_2O$ to the water added to the casing layer on two occassions between pinning and the first flush. This increased the copper in the casing about 100% (4 to 8 ppm). One crop (#2111) had copper added to half the trays at spawning by addition of 50 ml of a 4.2% solution of $CuSO_4 \bullet 5H_2O$ per tray prior to mixing in the spawn. This increased the copper content of the compost by about 100% (30 to 58 ppm, d.w.).

Mushrooms were harvested from the peak day of each flush of each prior to the normal harvest for laboratory analysis of quality and evaluation of shelf life using methods described earlier (Simons and Beelman 1995). The remainder of the mushrooms from each flush were shipped to the manufacturer for isolation of tyrosinase. Total tyrosinase activity present in all the mushrooms from each flush and treatment were



reported relative to the enzyme activity obtained per pound of the control treatment mushrooms from the first flush of Crop #2006 (normal compost and no copper added to the casing) reported as 100 units.

Mushrooms used for initial color measurements were removed immediately after the selective harvest of the peak day mushrooms. Three lots of eight mushrooms were randomly removed and used for color measurements. Three color readings were taken on each mushroom cap using a Minolta Chromameter (Model CR-200, Minolta Corp., Ransey, NJ) giving a total of 24 color readings for each replication of each treatment.

Mushrooms randomly selected from those harvested on the peak production day were evaluated for changes in color (browning) during storage as a means to evaluate the treatments on shelf life. Approximately 100g of mushrooms were placed into a linear polystyrene till (Tray-Pak Corp., Reading, PA). The tray was then overwrapped with polyvinylchloride film (60 gauge, PWMF Vitafilm, The Goodyear Tire & Rubber Co., Akron, OH) and a mild heat treatment was applied to ensure a proper seal. Two 3-mm holes were made through the film in opposite corners of the package to ensure an aerobic environment was maintained during postharvest storage. All of the packages were then stored in a 12°C environmental chamber (Lunaire Environmental, Inc., Williamsport, PA). Three packages were then sampled for color on days 0, 3, 6, and 9 of storage to evaluate treatment effect on shelf life color (Lab). Three L-value measurements were taken on the surface of each mushroom cap for all of the mushrooms in the package. The L-values for each package were then averaged by the Chromameter to produce one replicate L-value for each day of storage. The L-values obtained from each day of storage were then averaged to represent the browning (decline in L-value) that occurred during storage.

Each of the 24 mushrooms used for the initial color determination were quartered and one quarter of each mushroom was saved. Four randomly-selected quarters from four different mushrooms were placed together in an aluminum weigh dish (Fisher Scientific, Inc., Pittsburgh, PA) and frozen at -20°C. The frozen samples were freeze dried (Model VirTis 15-SRGX, VirTis, Inc., Gardiner, NY) until constant weight was obtained. Three freeze-dried mushroom samples taken from each treatment of each flush were sent to the Agricultural Analytical Services Laboratory at University Park, PA for mineral (copper) analysis. The standard method for the analysis of vegetables was used in preparing the samples before analysis of mineral content using atomic emission spectroscopy.

## 3 RESULTS AND DISCUSSION

Data indicating the influence of compost moisture and copper addition to the casing on crop yield and the copper content and initial whiteness of harvested mushrooms in Crop #2006 is presented in Table 1. The data indicated that the treatments only had modest effects on yield or copper content or whiteness (L-value) of the mushrooms at harvest. However, the trend of increased copper accumulation and decline in color (lower L-value) with each succeeding flush of the crop cycle was evident and was similar to the same trends observed previously in crops at the MTDF by Beelman *et al.* (1995). This decline in initial whiteness (L-value) of mushrooms at harvest with each succeeding flush of the crop cycle at the MTDF is a normal pattern (Bartley *et al.* 1991, Mau *et al.* 1993).

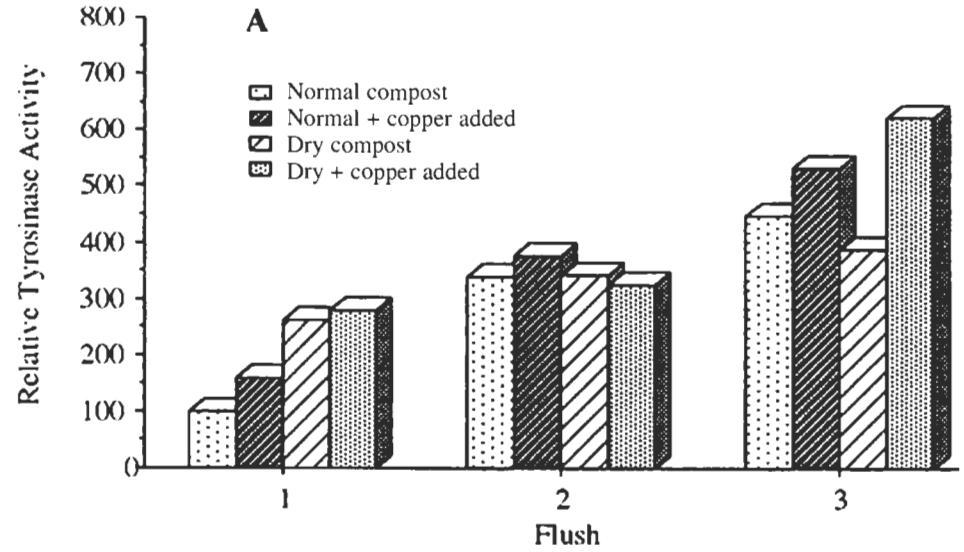
Table 1. Influence of compost moisture and copper added to the casing layer (Crop #2006) on yield and qualtiy attributes of traditional off-white mushrooms. Data are means of three replicate analyses within an individual flush. Means from the same attribute within each flush followed by the same letter are not significantly different. ($p<0.05$).

| Flush | Normal | | | Normal+copper | | | Dry | | | Dry+copper | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bed yield (lbs/ft²) | Copper (ppm; dw) | Initial whiteness (L) | Bed yield (lbs/ft²) | Copper (ppm; dw) | Initial whiteness (L) | Bed yield (lbs/ft²) | Copper (ppm; dw) | Initial whiteness (L) | Bed yield (lbs/ft²) | Copper (ppm; dw) | Initial whiteness (L) |
| 1 | 3.2 | 24A | 91.8A | 2.9 | 24A | 90.9A | 2.2 | 28A | 91.5A | 2.5 | 26A | 90.8A |
| 2 | 1.6 | 54A | 89.7B | 1.4 | 54A | 89.3B | 1.4 | 45AB | 90.9A | 1.5 | 44B | 89.0B |
| 3 | 0.7 | 58A | 88.6B | 0.5 | 63A | 88.6B | 0.5 | 60A | 90.4A | 0.4 | 68A | 89.6AB |
| Mean/ Total | 5.5 | 45 | 90.0 | 4.8 | 47 | 89.6 | 4.1 | 44 | 90.9 | 4.4 | 46 | 59.5 |

Relative tyrosinase activity in mushrooms from Crop #2006 varied by over 6-fold and was most affected by flush (Fig. 1A); it increased with each succeeding flush of the crop cycle as did the postharvest browning (decrease in L-value in storage) of the mushrooms (Fig. 1B). In most cases, relative tyrosinase was also increased by dry compost and addition of copper to the casing. Using the data in Fig. 1, a negative correlation of relative tyrosinase activity and decline of whiteness (L-value) of mushrooms in storage (browning) is illustrated in Fig. 2. Also, a positive correlation between copper content of mushrooms and relative tyrosinase activity is evident in Fig. 3.

Previous research conducted at the MTDF (Bartley *et al.* 1991) has demonstrated that whiteness (L-value) at harvest declined with mushrooms from each succeeding flush of the crop cycle and were more prone





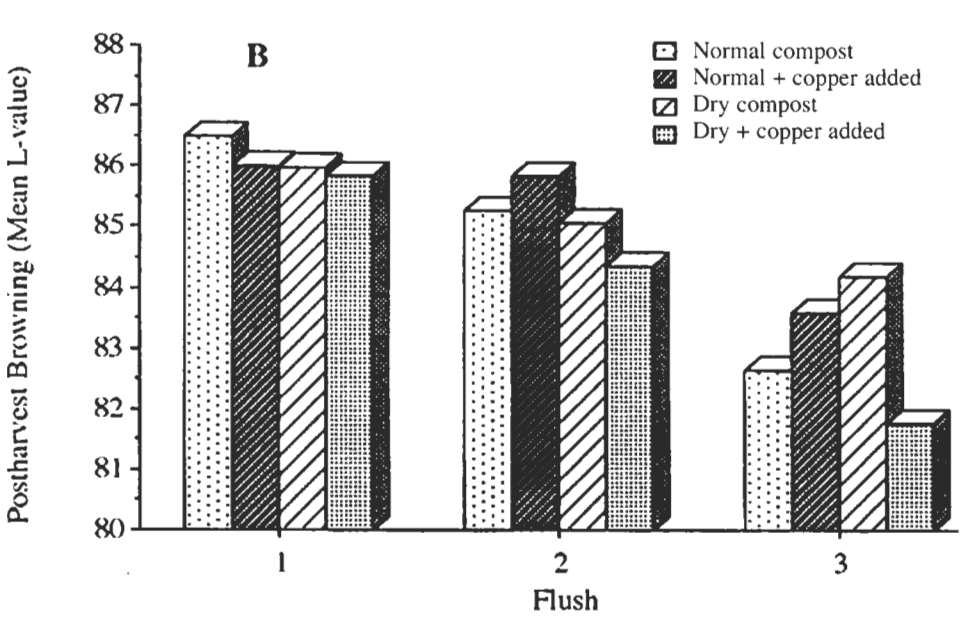




Fig. 1. Influence of compost moisture and copper added to the casing on relative tyrosinase activity (A) and browning during postharvest storage (B) of mushrooms from crop #2006.

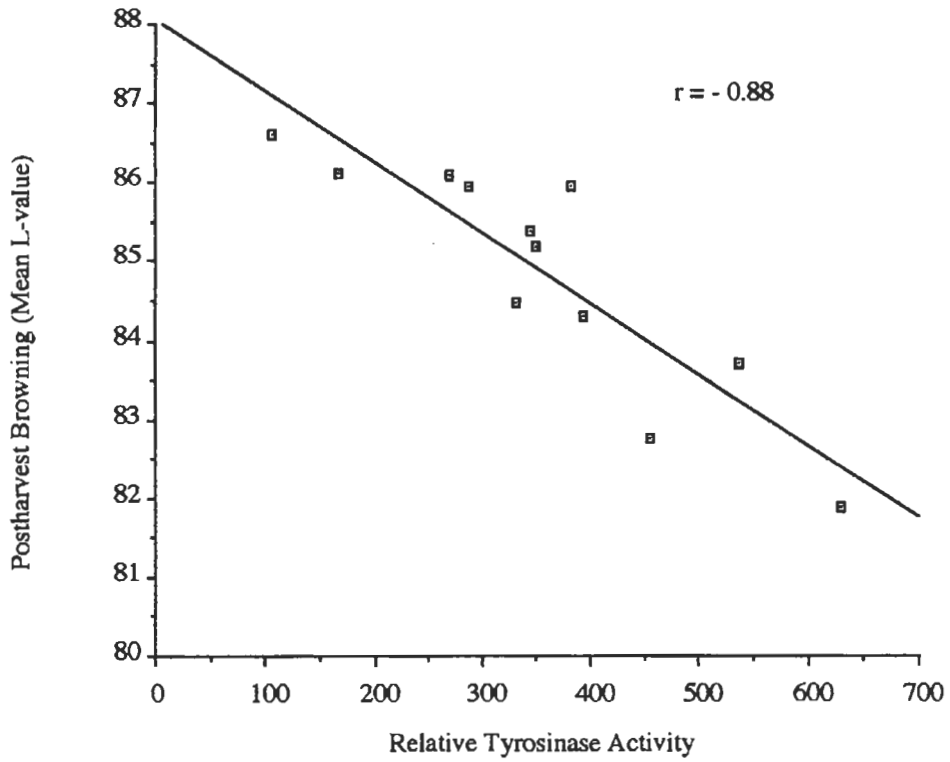



Fig. 2. Correlation of postharvest browing and relative tyrosinase activity in crop #2006.

to postharvest browning. The data reported herein indicate this could be due, at least in part, to increased tyrosinase activity. However, the data only reflect relative amounts of enzyme which cauld be isolated after several days of storage prior to isolation. Previous research demonstrated that tyrosinase increased in storage but differences in tyrosinase activity with mushrooms from different flushes were not evident (Burton 1988).

Similar results to those obtained from Crop #2006, were found in a replicate crop grown later (Crop #2017) but data are not presented. Effects of the same cultural treatments on yield, copper content and whiteness of the mushrooms were similar. Total relative tyrosinase activity that was recovered from the entire crops were also very similar (Fig. 4). However, in a third crop (#2111), where additional copper was added to the compost (2 times normal), much greater total relative tyrosinase activity (504 vs. 261 and 232) was obtained (Fig. 4). Surprisingly, the copper accumulated by the mushrooms was not significantly increased by the addition of copper to the compost (data not shown). This observation



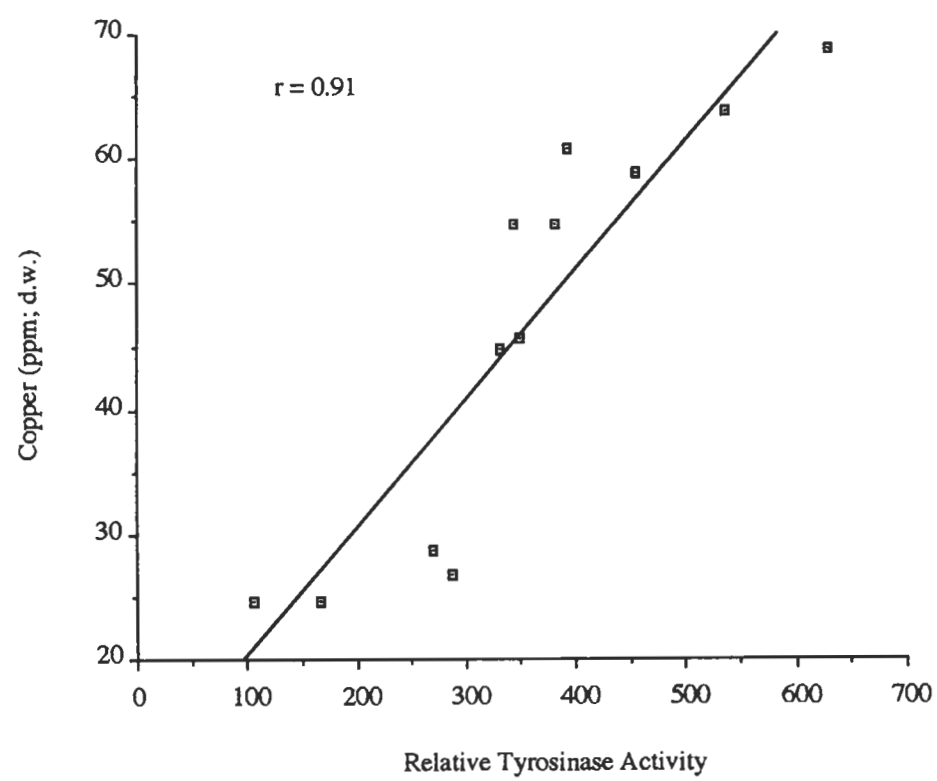



Fig. 3. Relationship between copper content and relative tyrosinase activity of mushrooms from crop #2006.

may indicate that the increase in relative tyrosinase activity was caused by a stress response to the increased level of copper in the compost, or some other factor. Regarding copper stress, Gruhn and Miller (1991) demonstrated that tyrosinase level of ectomycorrhizal fungi increased due to stress of excess copper (60 ppm) in their growth media. They suggested that the increase in tyrosinase synthesized may act as a detoxification mechanism by irreversibly binding copper ions to the enzyme. Previous work in our laboratory (Beelman *et al.* 1995) has demonstrated that mushrooms obtain most of their copper from the compost and increased levels in the compost can cause stress as evidenced by yield decreases. Hence, this area appears to warrant further experimentation.

The primary focus of this research was to determine cultural practices to increase tyrosinase activity of mushrooms for chemical isolation as a valuable product of commerce. However, the results should also be useful to those interested in improving quality and shelf life of mushrooms for food use.

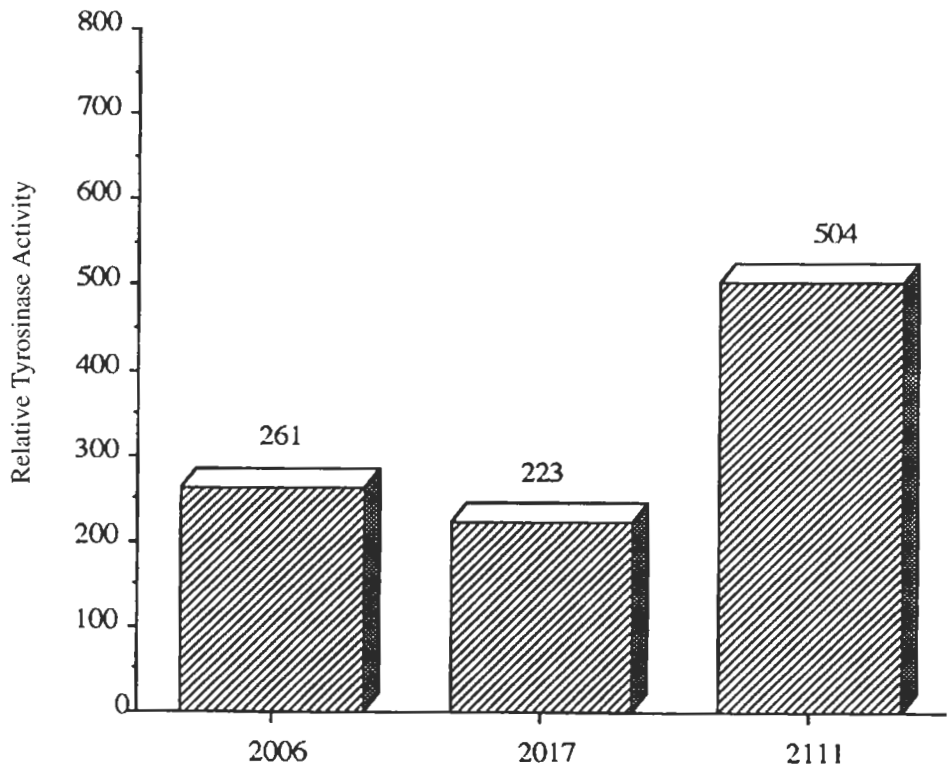



Figure 4. Total relative tyrosinase activity in mushrooms from two mushroom crops with copper added to the casing in one half of the trays (#2006 and #2017) and one crop (#2111) with copper added to the compost in one half of the trays.

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