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Vitamin D_2 Formation from Post-Harvest UV-B Treatment of Mushrooms (*Agaricus bisporus*) and Retention During Storage

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The objectives of this research were to study the effects of high intensity (0.5, 0.75, and 1.0 mW/cm^2), dose (0.5, 1.0, and 1.5 J/cm^2), and postharvest time (1 and 4 days) on the vitamin D_2 formation in Portabella mushrooms (*Agaricus bisporus*) as a result of UV-B exposure, as well as the vitamin D_2 degradation in treated mushrooms during storage. Within each intensity application, dose had the largest effect where more exposure converted more vitamin D_2 from ergosterol. Similar dose across each intensity application resulted in similar vitamin D_2 concentration. Practical commercial production requires as short a treatment time as possible, and intensity was a major factor from this standpoint where the time it took to achieve a similar vitamin D_2 concentration for similar dose exposure was significantly reduced as intensity increased. By using an intensity of 1.0 mW/cm^2 at a dose of 0.5 J/cm^2, the concentration of vitamin D_2 produced was 3.83 μg/g dry solids of mushrooms in 8 min, whereas using an intensity of 0.5 mW/cm^2 at a dose of 0.5 J/cm^2, the concentration of vitamin D_2 produced was 3.75μg/g dry solids of mushrooms in 18 min. Also, postharvest time did not have a significant effect on vitamin D_2 formation in mushrooms that were treated 1 and 4 days after harvest. Vitamin D_2 degraded in treated mushrooms during storage by apparent first-order kinetics, where the degradation rate constant was 0.025 h^{-1}. The information provided in this study will help mushroom producers develop commercial-scale UV treatment processes to add value to their crop while improving consumer health.

KEYWORDS: Ultraviolet; *Agaricus bisporus*; mushrooms; vitamin D; intensity; dosage; degradation

INTRODUCTION

More than 40% of American adults are deficient in Vitamin D (*1*). Vitamin D is important for calcium absorption and bone health, and Vitamin D deficiency can lead to softening of the bone in children and adults as well as osteoporosis in adults. Moreover, vitamin D has recently been linked to a significantly reduced risk of breast cancer, colon cancer, prostate cancer, autoimmune disease, and cardiovascular disease (*2*). Vitamin D intake comes naturally from sunlight and a limited number of foods. Dependency of sunlight as a vitamin D source is compromised for those avoiding sunlight or using sun block to reduce the chances of skin cancer (*2, 3*), those with dark skin where melanin acts like a sun block inhibiting UV light from penetrating deep enough in the skin for vitamin D to be produced, and those who are elderly in living-assistance and nursing homes and do not get enough sunlight and are at risk for vitamin D deficiency (*4*). Natural food sources for vitamin D are limited to a few animal sources, such as some fish and fish liver oils. A few foods, such as milk, cereal, and some fruit juices are fortified with vitamin D; however, additional natural food sources are needed to help the public meet the required daily intake of vitamin D.

Mau et al. showed that UV-B exposure of *Agaricus bisporus* mushrooms produced 12.48 μg of vitamin D_2/g of dried solid after a 2 h exposure time (*5*). This long time is impractical for commercial production and is likely due to the low intensity of UV-B used in the study, which was only 0.14 mW/cm^2. Extensive research has been conducted by Perera's group to maximize the conversion of ergosterol to vitamin D_2 using UV treatment on a variety of mushrooms (*6–8*); however, in each of these studies, they partially dried the mushrooms down to 78−80% moisture (wet basis) and then applied UV at an elevated temperature of 35 °C. Such conditions are not desirable to mushroom producers selling fresh or minimally processed mushrooms, such as washed and sliced. These next research studies provide some insight to more practical and ideal processing conditions to UV treat mushrooms for the fresh and minimally processed market. Mau et al. (*5*) showed that UV-B (310 nm) formed vitamin D_2 at a greater rate than from using

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UV-C (254 nm) in *A. bisporous* and *A. bitorquis* even though the intensity of UV-B (0.14 mW/cm^2) was less than UV-C (0.2 mW/cm^2); hence, the doses of UV-B were less. Jasinghe and Perera (*7*) showed application of UV-B to convert more erosterol to vitamin D_2 than UV-A or UV-C; however, comparison is compromised, since the UV source intensities were different and the treatment times were similar; hence, the doses were different. Jasinghe and Perera (*6*) did show the importance of orientation of mushrooms toward the UV source, where the gill-side of the mushrooms have greater concentration of ergosterol than the button-side; thus, more vitamin D_2 would be formed with the gill-side of the mushrooms facing the UV source. Two parameters that have not been studied are whether the time after harvesting the mushrooms before UV treatment has an effect and also the degradation kinetics of vitamin D_2 formed in mushrooms during storage.

The objectives of this research are (1) to investigate high intensity UV-B treatment on the formation of vitamin D_2 to determine whether formation of vitamin D_2 can be accelerated analogous to high temperature−short time, (2) to determine whether using various intensities will result in similar erosterol to vitamin D_2 conversion at similar doses, (3) to determine whether postharvest time of mushrooms before UV treatment has an effect on the conversion of ergosterol to vitamin D_2, and (4) to determine the vitamin D_2 retention/degradation in mushrooms treated with UV during normal storage conditions.

MATERIALS AND METHODS

Portabella *Agaricus bisporus* mushrooms were obtained from Monterey Mushrooms, Inc. (Watsonville, CA) in February and March, 2007. On the day that the mushrooms were picked up, they were either harvested that morning or 3 days prior; thus, when the mushrooms were treated with UV-B the next day, they were either 1 day postharvest or 4 day postharvest. The diameter of the mushrooms ranged between 4.6 and 5.2 cm. The mushrooms were stored in refrigeration at 2.2 ± 1.0 °C overnight before treatment. The mushrooms were taken out of refrigeration 4 h before treatment to bring the mushroom temperature up to room temperature (20 °C). Moisture content was determined by the vacuum oven method (*9*).

Experimental Design. In order for UV treatment to be practical for commercial-scale production, the treatment times need to be much less than the 2 h exposure time reported by Mau et al. (*5*). Thus, experiments were conducted to investigate vitamin D_2 formation at higher intensities (0.5, 0.75, or 1.0 mW/cm^2). In order to properly compare vitamin D_2 formation between each intensity, the time of exposure was varied for each intensity to obtain equivalent doses 0.5, 1.0, or 1.5 J/cm^2, which is simply calculated by multiplying intensity (W/cm^2) by time (s). The time lapse between when mushrooms are harvested to being treated with UV was also investigated, which would give information to mushroom producers as to a time frame when mushrooms need to be treated with UV. Thus, postharvest times 1 and 4 days postharvest were chosen, with 4 days postharvest being the maximum time mushrooms are stored before being shipped (*10*). Storage studies were investigated to determine vitamin D_2 retention in mushrooms after UV treatment. After mushrooms were treated, they were placed in foam containers (10−12 mushrooms per pack) and wrapped in perforated plastic. These containers and wrap were provided by Monterey Mushrooms, Inc. to replicate commercial mushroom packing. The mushrooms were then placed in refrigeration at 2.2 ± 1.0 °C for 2 and 4 days. The storage study had two parts: (1) quantifying the degradation kinetics of vitamin D_2 in 1 day postharvest mushrooms treated at an intensity of 0.75 mW/cm^2 for doses of 1.0 and 1.5 J/cm^2 and stored at 0, 2, and 4 days and (2) comparing the degradation of vitamin D_2 between 1 and 4 day postharvest mushrooms both treated using an intensity of 0.75 mW/cm^2 for doses of 1.0 and 1.5 J/cm^2 and stored at 0 and 2 days, which was the maximum storage for 4 day postharvest mushrooms.

UV Treatments. The UV unit (Research Unit, The Daavlin Company, Bryan, OH) was used, equipped with 8 UV-B (305 nm) lamps 121.9 cm (48 in.) in length; thus, the total treatment area was 0.81 m^2. The intensity output was adjusted by adjusting the height of the unit, which the bulb-to-bench height ranged 15.2−45.7 cm (6−18 in.). Intensity was measured using a radiometer/photometer (Model 1400A, International Light, Peabody, MA) with a UV-B filter (#2209) and an input UV-B calibration factor of 1.811×10^{-4}. The UV unit lamp-to-sample height was adjusted until the desired intensity of 0.5, 1.0, or 1.5 mW/cm^2 was reached. The mushrooms were treated in 1 kg batches, which was approximately 49−52 mushrooms occupying an area of 0.13 m^2. Immediately after treatment, the mushrooms were placed in labeled storage bags and chilled in a cooler of dry ice. For vitamin D_2 analysis, mushrooms need to be freeze-dried, milled, and packed in nitrogen-flushed Mylar pouches. Once all of the UV treatments were complete, mushrooms were placed into a walk-in freezer room overnight. Freezing the mushrooms prior to loading them into the freeze-drier reduces the load on the freeze-drier and the overall drying time. The nine tray freeze-dryer (Ultra 25EL, VirTis (an SP Industries Company), Gardner, NY) was able to freeze-dry up to 15 kg of mushrooms. Once the mushrooms were completely dry, each batch was milled using a hand-held blender (Braun, MR 5550 CA, 400 W, hand-blender, Gillette Commercial Operations, Boston, MA). The milled mushroom powder was then packed in a metallized Mylar pouch, flushed with nitrogen, and sealed ready for shipment.

Vitamin D_2 Analysis. The packages of mushroom powder were shipped to Medallion Laboratories (Minneapolis, MN) for vitamin D_2 analysis. The analysis was based on the standard method 2002.05 (*11*). Dihydrotachysterol (an internal standard), ascorbic acid, and pyrogallic acid were added to the sample, and the mixture was saponified with an ethanol/KOH solution under nitrogen. The vitamins and internal standard were extracted into heptane, evaporated to dryness, and reconstituted into 1:1 cyclohexane/heptane. The solution underwent preparative HPLC cleanup (NucleoSil 100 silica column 4.6 mm × 250 mm, mobile phase 0.5% isopropanol/2% methyl-tert-butyl-ether in 1:1 cyclohexane/heptane, detection at 260 nm). The fraction containing the internal standard and vitamin D_2 were collected, dried under nitrogen, and reconstituted in 12% methanol/88% acetonitrile. Quantitation was performed by reverse-phase HPLC with mass spectrometry detection (VyDac 218TP54 C18 column, 4.6 mm × 250 mm, mobile phase 12% methanol/88% acetonitrile, detection via mass spectrometry, Water Quattro Premier XE Micromass). The reported standard deviation for this method was 4.7% (*12*).

Statistical Analysis. The vitamin D concentrations were expressed as means ± SD (standard deviation). The results were statistically analyzed by analysis of variance (ANOVA), and the means were compared using Least Significant Difference (LSD) with $p \leq 0.05$. Both the ANOVA and LSD tests were conducted using Excel software.

RESULTS AND DISCUSSION

Table 1 shows the results of the UV-B exposure treatment of mushrooms with respect to intensity, dose, and postharvest time. The moisture content of mushrooms was 90.2 ± 0.5% (wet basis); thus, based on an 84 g serving of mushrooms (*13*), the dry solids (d.s.) weight would be 8.23 g. **Table 1** shows quantities of vitamin D_2 with respect to micrograms per gram d.s., micrograms per serving, and percent daily value (DV) based on 10 μg (*14*). With exception of the 4 day postharvest mushrooms treated at an intensity of 1.0 mW/cm^2 for a dosage of 0.5 J/cm^2, there was no significant difference ($p = 0.05$) in vitamin D_2 generation between 1 and 4 day postharvest mushrooms. Within each intensity condition, the dosage was clearly significant where the greater the dosage the more vitamin D_2 was generated, as reported by Mau et al. (*5*). The vitamin D_2 generation appears to follow first-order kinetics. Previous studies showed a linear relationship between vitamin D_2 formation and dosage where the formation rate for UV-B treatment was 5.04 μg/h and the maximum vitamin D_2 concentration formed was 12.48 μg/g of dry mushrooms in *A. bisporus* (*5*). As shown in **Table 1**, the rate of formation is much greater than 5.04 μg/h because of the much greater intensities used in

Table 1. UV-B Treatment of Mushrooms Comparison at Three Intensities, Three Doses, and Treated 1 and 4 Days after Harvest[1]

dose	time	μg/g d.s.		μg/serving[2]		% DV (10 μg)	
(J/cm²)	(min)	1 day	4 day	1 day	4 day	1 day	4 day
Intensity = 0.46 mW/cm²							
0	0	0.01	0.01	0.07	0.07	0.67	0.67
0.5	18.1	3.75 ± 0.18^{g,h}	3.55 ± 0.17^{h}	30.87	29.22	308.7	292.2
1.0	36.2	4.53 ± 0.21^{f}	4.30 ± 0.20^{f,g}	37.25	35.40	372.5	354.0
1.5	54.4	7.28 ± 0.34^{b}	7.20 ± 0.34^{b}	59.89	59.27	598.9	592.7
Intensity = 0.75 mW/cm²							
0	0	0.01	0.01	0.07	0.07	0.67	0.67
0.5	11.1	3.48 ± 0.16^{h}	3.35 ± 0.16^{h}	28.61	27.58	286.1	275.8
1.0	22.2	5.58 ± 0.26^{d,e}	5.33 ± 0.25^{e}	45.89	43.84	458.9	438.4
1.5	33.3	7.2 ± 0.34^{b}	7.08 ± 0.33^{b}	59.27	58.24	592.7	582.4
Intensity = 1.0 mW/cm²							
0	0	0.01	0.01	0.07	0.07	0.67	0.67
0.5	8.3	3.83 ± 0.18^{g,h}	4.43 ± 0.21^{f}	31.49	36.43	314.9	364.3
1.0	16.7	6.2 ± 0.29^{c}	6.05 ± 0.28^{c,d}	51.04	49.80	510.4	498.0
1.5	25.0	7.43 ± 0.35^{a,b}	7.98 ± 0.38^{a}	61.12	65.65	611.2	656.5

[1] Different letters indicate a significant difference in descending order from "a" through "h" (least significant difference at $p \leq 0.05$). [2] Based on a serving of 84 g of fresh mushroom having a moisture content of 90.2 ± 0.5%.

this study, 0.5, 0.75, and 1.0 mW/cm² versus 0.14 mW/cm² in the study by Mau et al. (*5*). Also and more importantly from a commercial processing standpoint, the times it took to achieve 1 J/cm² dose in this study were 36.2, 22.2, and 16.7 min when using 0.5, 0.75, and 1.0 mW/cm², respectively, compared to 2 h using 0.14 mW/cm² in the study by Mau et al. (*5*). The lower final concentration of vitamin D_2 formed (**Table 1**) in comparison to Mau et al. (*5*) is probably because of the lower available ergosterol on the button side of the mushrooms, as was the orientation in this study, compared to the available ergosterol in the gill-side of the mushrooms (*5, 6*) Again, the reason the button-side orientation was used in this study was to present a worse-case scenario processing condition, since it is likely that as mushrooms are processed on a continuous belt through a commercial UV unit the mushrooms will be oriented either button-side or gill-side. Comparing the intensity of treatment within each dosage, the 1.0 J/cm² dosage shows more of a clear pattern where the higher intensity formed a higher concentration of vitamin D_2: 6.2 and 6.05 μg/g dry solids using 1.0 mW/cm² for 1 and 4 day postharvest, respectively; 5.58 and 5.33 μg/g dry solids using 0.75 mW/cm² for 1 and 4 day postharvest, respectively; and 4.53 and 4.3 μg/g dry solids using 0.5 mW/cm² for 1 and 4 day postharvest, respectively. However, at the lower and higher dosages, 0.5 J/cm² and 1.5 J/cm², intensity does not appear to be a significant factor in final vitamin D_2 concentration, which is expected, since similar dosages equate different intensity treatments by varying the time of exposure. There was no visible color differences between the mushrooms before and after UV-B treatment, as was reported by Mau et al. (*5*), and the temperature of the mushrooms remained at room temperature throughout the treatment.

Figure 1a shows the retention of vitamin D_2 in mushrooms exposed to 0.75 mW/cm² of UV-B for doses 1.0 and 1.5 J/cm² during storage at 2.2 °C. After 4 days of storage, there was still over 150% of the daily value of vitamin D_2 in a single serving of mushrooms. The vitamin D degradation appears to follow a first-order reaction:

$$\frac{d[C]}{dt} = -k[C] \quad (1)$$

where C is the concentration of vitamin D_2 (μg/g d.s.), t is time

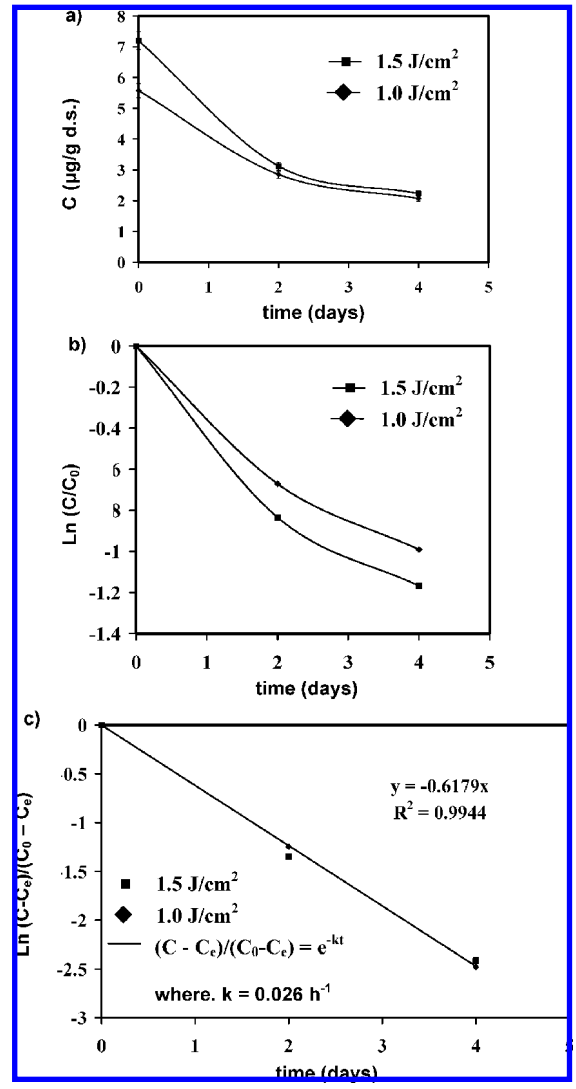

Figure 1. Vitamin D degradation in mushrooms treated using 0.75 mW/cm² intensity and doses of 1.0 and 1.5 J/cm².

(h), and k is the first-order rate constant (1/h). If the assumption that vitamin D_2 degrades completely to 0, then the solution of eq 1 is

$$\ln \frac{[C]}{[C_0]} = -kt \quad (2)$$

where C_0 is the concentration of vitamin D_2 at the initial time of storage (μg/g d.s.). If the degradation follows first-order kinetics, then a plot of $\ln({}^{[C]}/_{[C_0]})$ versus t, as shown in **Figure 1b**, should result in a straight line. However, **Figure 1b** shows that the vitamin D_2 degradation is not linear and suggests that either the assumption of vitamin D_2 not degrading completely but to some equilibrium value, C_e, or the degradation follows second-order kinetics. Typically, vitamin degradation does follow first-order kinetics, at least mathematically if not mechanistically (*15*), and the form of this equation is as follows:

$$\ln \frac{[C - C_e]}{[C_0 - C_e]} = -kt \quad (3)$$

The equilibrium or end-point concentration of vitamin D_2 was determined until a straight line was observed (**Figure 1c**), and this equilibrium value is 1.75 μg/g d.s. From the slope of the curve, the first-order rate constant was calculated as 0.026 h^{-1}. By using this rate constant in eq 3, the time it would take for

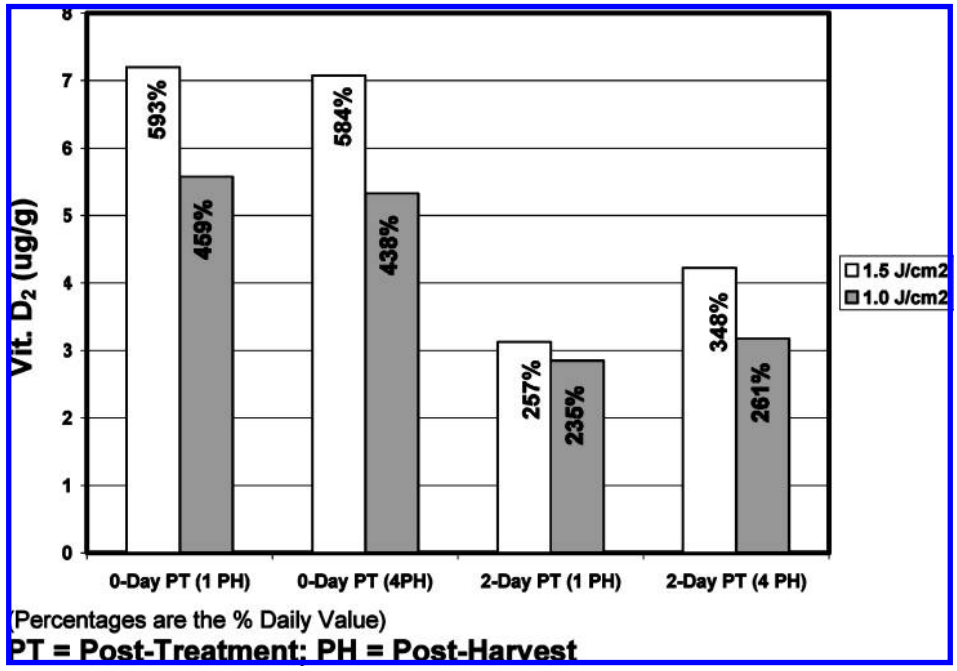

Figure 2. Vitamin D concentration in mushrooms treated using 0.75 mW/cm^2 intensity as a function of dose, postharvest time, and post-treatment storage.

vitamin D_2 in the mushrooms to degrade to 1.75 μg/g d.s. would be over 14 days, which is well beyond the shelf life of the mushrooms (*10*). On the basis of this information and the fact that using an end-point concentration provided a straight line to the first-order degradation of vitamin D, 1.75 μg/g d.s. is a good estimate of the end-point vitamin D_2 concentration at the time where the mushrooms degrade physically. Moreover, the very high R^2 value in **Figure 1c** shows a very good prediction of the vitamin D_2 degradation in the mushrooms during refrigerated storage.

Figure 2 shows the comparison study of vitamin D_2 generation and degradation between 1 and 4 day postharvest mushrooms treated using 0.75 mW/cm^2 intensity at doses of 1.0 and 1.5 J/cm^2 and after 2 days of refrigerated storage. In addition to providing important dose response data shown in **Table 1**, this study showed for the first time that there is no difference in vitamin D_2 production between mushrooms treated 1 day after being harvested versus those treated 4 days after being harvested. In addition, this is the first study to demonstrate that there is no difference in the retention of vitamin D_2 after 2 days in refrigerated storage following UV treatment between 3 day old mushrooms (1 day postharvest + 2 day storage) and 6 day old mushrooms (4 day postharvest + 2 day storage). The greatest concentration of vitamin D_2 generated and retained was in the mushrooms treated at the higher dosage of 1.5 J/cm^2.

UV light equipment is inexpensive and offers the mushroom industry an innovative and affordable means to significantly increase the value of their mushrooms. This study provides mushroom producers useful processing information (high intensity−short time and vitamin D degradation during storage) as well as the flexibility after harvesting between 1 and 4 days to treat mushrooms with UV.

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