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Effects of Postharvest Pulsed UV Light Treatment of White Button Mushrooms (*Agaricus bisporus*) on Vitamin D_2 Content and Quality Attributes

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ABSTRACT: Pulsed UV light (PUV) was investigated as a means to rapidly increase vitamin D_2 (D_2) content in fresh button mushrooms (*Agaricus bisporus*). D_2 was found to increase to over 100% RDA/serving following 3 pulses (1 s). Following 12 pulses, D_2 began to approach a maximum concentration of 27 μg/g DW. The D_2 produced with 3 pulses decreased from 11.9 to 9.05 μg/g DW after 3 days of storage; however, D_2 levels remained nearly constant after this point throughout an 11-day shelf life study. PUV treated sliced mushrooms produced significantly more D_2 than whole mushrooms, and it was also observed that brown buttons generated significantly less D_2 than white buttons. Several quality attributes were assessed, and no significant differences between control and PUV treated mushrooms were observed. These findings suggest that PUV treatment is a viable method for rapidly increasing the D_2 content of fresh mushrooms without adversely affecting quality parameters.

KEYWORDS: *vitamin D_2, mushrooms, pulsed ultraviolet light*

INTRODUCTION

Vitamin D deficiency has recently become a major public health issue. Current research suggests that inadequate intake of vitamin D results in more than rickets and osteomalacia, a consequence of inadequate bone mineralization.[1] Links to vitamin D deficiency and diseases such as cardiovascular disease[2] and cancer[3] have been documented. Other diseases with links to vitamin D deficiency include hypertension, stroke, diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, periodontal disease, macular degeneration, mental illness, and chronic pain.[4]

Humans are capable of converting cholesterol present in the epidermis to vitamin D_3 (cholecalciferol) following sun exposure.[5] Individuals with limited sun exposure are most at risk for vitamin D deficiency. Other groups at risk include infants who are exclusively breastfed, the elderly, obese individuals, and those with dark skin pigmentation.[1] Apart from sun exposure and dietary supplements, vitamin D can be acquired through the diet, although few foods contain sufficient endogenous levels. These include fatty fish (e.g., salmon and mackerel), eggs, and bovine liver. Fortified foods (e.g., milk, orange juice, and cereals) are, therefore, an important source of vitamin D in the American diet. Vitamin D_3 used for fortification is typically produced from cholesterol obtained from lanolin, an animal derived product, and is not considered vegan.[6] Mushrooms, however, represent a potential source of vitamin D that is not derived from animals and, therefore, can be considered vegan.[7]

Mushrooms contain high levels of pro-vitamin D_2 (ergosterol), and studies have shown that some wild mushroom species contain naturally occurring levels of vitamin D_2, ranging from 2.91−58.7 μg/100 g fresh weight.[8−10] This most likely results from sun exposure of wild mushrooms, which promotes the conversion of endogenous ergosterol to ergocalciferol (vitamin D_2). The vitamin D_2 content of mushrooms can also be enhanced through the use of artificial UV light.[9,11−19] These studies have shown that exposure to UV light can increase vitamin D_2 content in mushrooms from nondetectable levels to over 100 μg/g dry weight. The vitamin D_2 produced in UV irradiated mushrooms has been shown to be biologically active in humans.[20]

The use of continuous UV irradiation to increase vitamin D_2 content, however, is not without its limitations. The exposure time required to generate adequate amounts of vitamin D_2 is on the order of minutes to hours, which may be too long to be practical for mushrooms produced under commercial conditions. Significant browning of white button mushrooms exposed to continuous UV has also been reported,[9,11,17] which is a serious issue with respect to consumer acceptability.

In contrast of continuous UV, pulsed UV light (PUV) is a technology that utilizes a broad spectrum (100−800 nm) lamp along with high intensity pulses, delivering high amounts of energy in a relatively short amount of time. Compared to continuous UV light systems, the time required to deliver the same dose of UV irradiation is much shorter with PUV.[21] In addition, continuous UV systems do not typically employ broadband lamps and are only capable of encompassing one of the three principal regions of the UV spectrum: UV-A (400−315 nm), UV−B (315−280 nm), and UV−C (280−100 nm). The use of a broadband PUV system for vitamin D_2 enrichment could prove to be more attractive for commercial use due to the decreased exposure times and the potential for higher throughput.

Previous research conducted in our laboratory[22] demonstrated that PUV, using a C-type lamp (encompassing all

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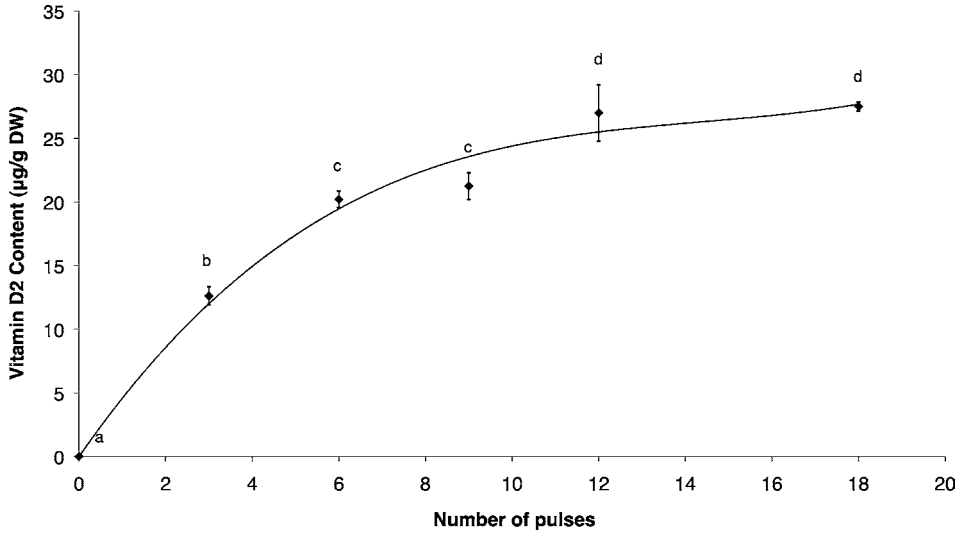

Figure 1. Vitamin D_2 content of sliced fresh white button (*Agaricus bisporus*) mushrooms treated with pulsed UV light in increments of 3 pulses (3 pulses = 1 s exposure time). Error bars represent standard deviations. Mean values with lower case letters that are the same are not significantly different ($p < 0.05$).

regions of the UV spectrum amplified in the UV−C region), could be used to rapidly produce vitamin D_2 in fresh mushrooms.

The present study was conducted to determine methods needed to optimize PUV treatment using a B-type lamp (UV−C wavelengths excluded) to produce significant amounts of vitamin D_2 in fresh mushrooms following very short exposure times, as well as to study the factors influencing the amount of vitamin D_2 produced. A dose−response study was also carried out, in part to determine the maximum yield of vitamin D_2 obtainable by PUV. The effects of our PUV process on the shelf life of fresh mushrooms and their quality attributes were evaluated. The retention of the vitamin D_2 produced by this process during postharvest storage was also determined.

MATERIALS AND METHODS

Materials. Mushrooms (*Agaricus bisporus* (J.E. Lange) Imbach) were obtained from the Pennsylvania State University Mushroom Test and Demonstration Facility on the day of harvest and were treated the same day. All mushrooms were protected from light exposure throughout the experiments. Materials for microbial enumeration included buffered peptone water (BPW), plate count agar (Standard Methods Agar), and dichloran-rose bengal-chloramphenicol agar (DRBC) (BD Difco, Sparks, MD).

Dose−Response Study. To determine the dose−response of PUV treatment to vitamin D_2 production in fresh mushrooms, white button mushrooms (*Agaricus bisporus*) were cut into 7 mm slices and weighed into 150 g lots in polystyrene containers. A Steripulse-XL 3000 (Xenon Corporation, Woburn, MA) was used with a B-type lamp for PUV experiments. The lamp was situated 5.8 cm from a quartz window. The apparatus generated 3 pulses/s at 505 J/pulse. At 3.18 cm from the quartz window, the broadband energy was 0.791 J/cm^2/pulse. Mushroom samples were treated in increments of 3 pulses from 0 to 18, at a distance of 3.18 cm from the quartz window, in order to simulate treatment in a commercial package.

Mushroom Variety, Slicing, and Package Weight. White and brown button (*Agaricus bisporus*) mushrooms were treated as described above with 3 pulses, either as whole mushrooms or 7 mm slices. White button mushrooms were also weighed into 150 and 230 g weight packages to determine the effect of the amount of mushrooms treated in a single package on vitamin D_2 generation.

Shelf Life Study and Vitamin D Retention Analysis. White button mushrooms were sliced and treated in 150 g lots with either 0 or 3 pulses of PUV. Packages were subsequently overwrapped with PVC film, and three holes were punched in the top of each package to replicate commercial packaging. Mushrooms were stored at 3 °C to replicate storage conditions under refrigeration. Whole package samples were taken during storage at days 0, 3, 6, and 11 and tested for vitamin D_2 content and quality attributes.

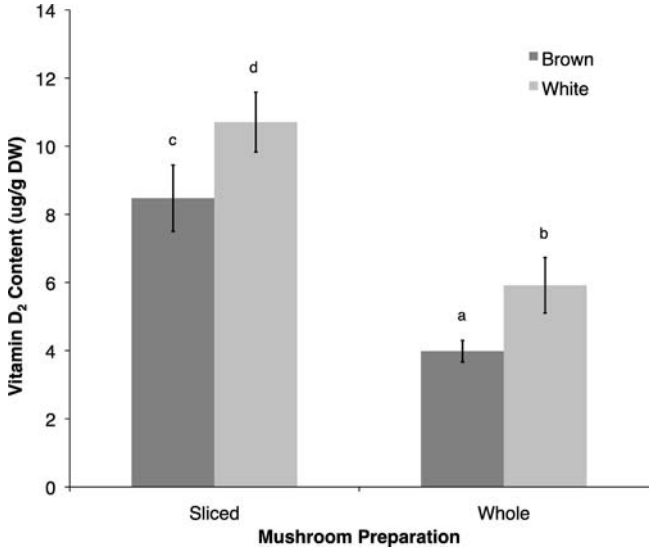

Figure 2. Vitamin D_2 content of sliced fresh white and brown button (*Agaricus bisporus*) mushrooms treated with pulsed UV light (3 pulses = 1 s exposure time; control mushrooms received 0 pulses) in either sliced or whole form. Mean values with lower case letters that are the same are not significantly different ($p < 0.05$). (Control mushrooms contained 0.0106 and 0.265 μg/g in brown and white varieties, respectively.)

Microbiological Analysis. The microbiological population of PUV treated mushrooms was analyzed on days 0 and 11 of the shelf life study. Samples (150 g) were transferred to a Waring LB10S laboratory blender and diluted 1:1 with buffered peptone water (BPW) and homogenized for 30 s. Following serial dilution with BPW, samples were plated on Standard Methods Agar and DRBC agar for analysis of aerobic plate count and yeasts and molds, respectively. The inoculated plates were incubated under aerobic conditions at 25 °C for 48−92 h after which colonies were enumerated.

Quality Assessment. Three markers of quality were assessed to determine differences between PUV treated and untreated mushrooms. Weight loss, whiteness, and visual appearance were measured at days 0, 3, 6, and 11 of storage at 3 °C. To determine weight loss, weight changes were monitored over the course of storage, and percent weight loss was normalized to day 0 values. Whiteness was assessed in terms of L-value using a Minolta Chroma Meter (Model CR-200, Japan) (n = 20 measurements per sample).

Vitamin D_2 Analysis. Samples were snap frozen (−80 °C) directly following treatment or after a predetermined number of days of storage, depending on the experiment. Following lyophilization, freeze-dried mushrooms were homogenized and analyzed for vitamin D_2 content according to established methods.[16] Briefly, each mushroom sample was saponified with dihydrotachysterol (internal standard), ascorbic acid, and pyrogallic acid in an ethanol/KOH solution, as described previously.[16] The samples were then extracted using heptane, dried, and reconstituted in cyclohexane and heptane (1:1). The solution underwent preparative HPLC cleanup, and the fraction containing the internal standard and vitamin D2 was collected, dried under nitrogen, reconstituted in 1:1 cyclohexane/heptane and quantitated by reverse-phase HPLC with mass spectrometry detection. Vitamin D_2 values are reported as μg/g dry weight (DW).

Statistical Analysis. All experiments were performed in triplicate. One-way ANOVA and Tukey's test (α = 0.05) were performed using Minitab 15 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

The results of the dose−response study (Figure 1) suggest a nonlinear relationship between PUV irradiation dose and vitamin D_2 content of fresh mushrooms. The initial levels of

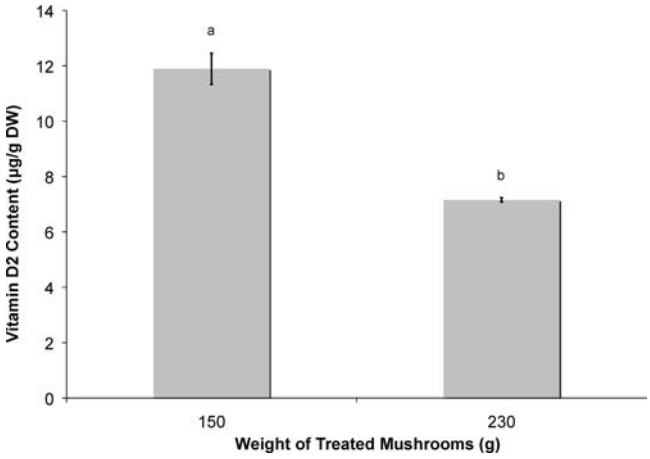

Figure 3. Vitamin D_2 content of sliced fresh white button (*Agaricus bisporus*) mushrooms treated in two different weight packages with pulsed UV light (3 pulses = 1 s exposure time). Mean values with lower case letters that are the same are not significantly different (p < 0.05).

vitamin D_2 in untreated mushrooms were less than 0.005 μg/g DW, and rapidly increased to 12.6 μg/g DW after 3 pulses. This is equivalent to 518% of the RDA of vitamin D per serving of fresh mushrooms (84 g fresh weight) based on the current RDA of 600 IU for vitamin D.[1] Vitamin D_2 levels in mushroom samples then appeared to level reach a maximum concentration of approximately 27 μg/g DW or 1130% RDA/serving between 12 and 18 pulses.

Jasinghe et al.[14] studied the kinetics of vitamin D_2 conversion from ergosterol in fresh mushrooms. D_2 production was observed to increase linearly as a function of exposure time to continuous UV irradiation, suggesting zero order kinetics.

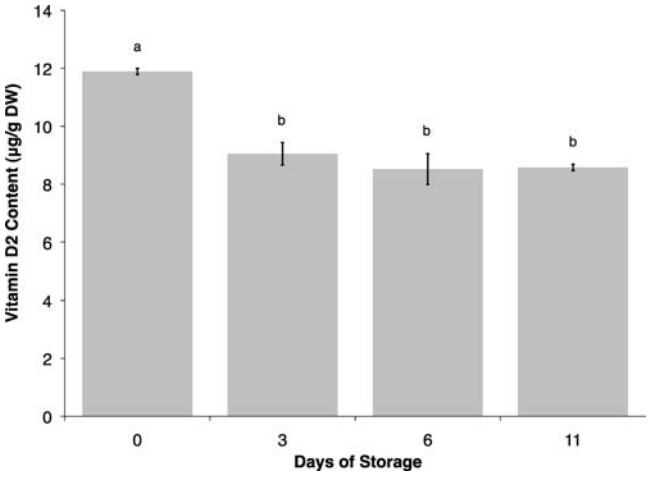

Figure 4. Vitamin D_2 content of sliced fresh white button (*Agaricus bisporus*) mushrooms treated with pulsed UV light (3 pulses = 1 s exposure time) and stored for up to 11 days at 3 °C. Mean values with lower case letters that are the same are not significantly different (p < 0.05).

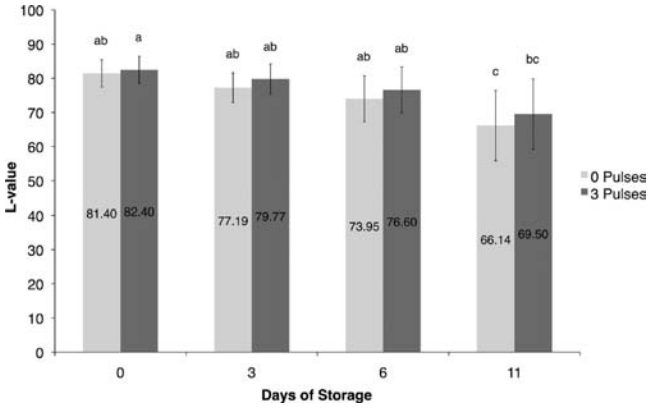

Figure 5. Whiteness (L-value) of untreated and pulsed UV light treated sliced fresh white button (*Agaricus bisporus*) mushrooms. L-values of 0 and 100 represent black and white, respectively. Mean values with lower case letters that are the same are not significantly different (p < 0.05).

However, our results indicate that the conversion of ergosterol to vitamin D_2 may not follow zero order kinetics after exposure to the relatively higher levels of UV irradiation used in our study. In a separate study, Jasinghe and co-workers reported similar kinetics in shiitake mushrooms treated up to 120 min with UV-A irradiation, wherein the maximum D_2 concentration obtained was ca. 30 μg/g DW with the gill tissue facing the lamp.[13] This phenomenon observed in our study could potentially be due to a low depth of penetration of UV irradiation due to the thickness of the batch of mushrooms being treated as opposed to a single layer system. It has also been suggested that photodegradation of D_2 may result with prolonged exposure.[23] In addition, side products such as lumisterol and tachysterol may be formed with prolonged exposure.[14]

For all the subsequent studies, a dose of 3 pulses was chosen to represent what would be a reasonable treatment in terms of both D_2 produced and ease of implementation in a commercial setting. The high level of D_2 produced, even with this one second treatment, allowed for the various other parameters to be evaluated at what would probably be the upper level of

Figure 6. Photograph of sliced fresh white button mushrooms after storage at 3 °C for 0 days (a), 3 days (b), 6 days (c), and 11 days (d). The top row of each quadrant represents untreated mushrooms; the bottom row of each quadrant represents mushrooms treated with 3 pulses of pulsed UV light.

Table 1. Percent Weight Loss in Pulsed UV Treated Sliced Fresh White Button (*Agaricus bisporus*) Mushrooms from Initial Weight (150 g) after Storage at 3 °C for up to 11 Days

	days of storage		
number of pulses	3	6	11
0	1.69 ± 0.62	3.42 ± 0.33	5.33 ± 0.24
3	1.87 ± 0.76	3.76 ± 1.18	6.19 ± 1.60

Table 2. Total Aerobic Plate Counts and Yeast and Mold Counts of Pulsed UV Treated (3 Pulses) and Untreated Sliced White Button (*Agaricus bisporus*) Mushrooms Directly Following Treatment and after 11 Days of Storage at 3 °C

	day 0		day 11	
	control (CFU/g)	treatment (CFU/g)	control (CFU/g)	treatment (CFU/g)
total aerobic plate count	6.21 ± 0.16	6.27 ± 0.19	8.74 ± 0.19	8.55 ± 0.22
yeasts and molds	5.88 ± 0.09	6.02 ± 0.34	8.21 ± 0.79	8.11 ± 0.18

commercial treatment. This treatment would therefore be the most rigorous commercial treatment, and any effects on browning, microbial inactivation, etc. would be visible. If no effects were seen with this treatment, any lower dose treatment (i.e., to achieve a D_2 content of 400 IU/serving or 100% of the daily value) would likely not suffer any benefits or consequences seen in a higher dose. Implementing a full range of doses to investigate the following parameters would have been beyond the scope of this study.

Brown and white button mushrooms were treated with 3 pulses of PUV in sliced and whole forms. There was significantly more vitamin D_2 in sliced mushrooms ($p < 0.05$) compared to that in whole in both brown (113% more) and white (81% more) mushrooms (Figure 2). This is most likely due to the increased exposure of the gill tissue in the sliced mushrooms. Ergosterol levels have been shown to vary in different mushroom tissues. Jasinghe and Perera[12] showed that shiitake mushrooms contained more ergosterol in the gill tissue than the cap while Ko et al.[15] demonstrated that treating the gill tissue of button mushrooms resulted in more vitamin D than when the pileus was treated. On the basis of our results and the previously mentioned ergosterol distribution studies, it is conceivable that ergosterol is concentrated within the gill tissue of the brown and white button mushrooms used in the present study. As slicing increases gill tissue exposure to the light source, one would expect higher final levels of D_2 in sliced versus whole samples. However, further research is needed to establish the ergosterol content of the various tissues of *Agaricus bisporus* and to determine if this phenomenon is simply a result of the exposed gill tissue or a surface area effect (i.e., the surface-area-to-volume of gill tissue is higher than that of the smooth cap). There was also significantly less ($p < 0.05$) D_2 in brown button varieties compared with that in whites in both the sliced (21% less) and whole (33% less) treatment groups. It is likely that the higher levels of pigmentation in the brown mushroom samples shielded endogenous ergosterol from the UV source.

The penetrating effect of PUV on mushroom samples was investigated within the context of D_2 production. When a full commercial package of mushrooms (230 g) was treated in a batch system, the final vitamin D_2 content (7.6 μg/g DW) was 40% lower ($p < 0.05$) than those treated in 150 g batches (11.9 μg/g DW) (Figure 3). These data suggest that the top layer of mushrooms were exposed to a higher proportion of UV light and, therefore, generated higher levels of D_2. On the basis of these results, it is suggested that PUV treatment of mushrooms in a single layer would result in a more even distribution of D_2 throughout all mushrooms.

A shelf life study of PUV treated mushrooms was carried out, where it was observed that 76% of the D_2 produced was retained in all samples throughout storage (Figure 4). The mushrooms had an initial level of 11.9 μg/g DW after treatment with 3 pulses of PUV. After 3 days of storage (3 °C), the level dropped by 24% to a final concentration of 9.05 μg/g DW; however, this level did not change significantly ($p < 0.05$) for the duration of the storage study. A previous study[16] found a similar initial decrease and subsequent leveling off of vitamin D_2 levels in mushrooms stored at 2.2 °C, although the study was only carried out for 4 days.

A visual quality assessment indicated no significant difference ($p < 0.05$) in untreated and PUV treated mushrooms: no discernible difference in whiteness and no overall difference in visual appearance between untreated and PUV treated white button mushrooms at any time point during storage through 11 days (Figures 5 and 6). Previous studies using continuous UV light have shown browning of white button mushrooms.[9,11,17] One study[15] also reported browning in addition to a decrease in moisture content with continuous UV treatment. PUV does not appear to yield the same deleterious effects; however, further research is needed to investigate the basis of this phenomenon. No significant difference ($p < 0.05$) in weight change of PUV treated mushrooms from day 0 through 11 days of storage was observed compared to that of the control (Table 1). Weight change is a measure of degradation of the mushroom during storage as moisture and other components are lost through desiccation. These results indicate that PUV treatment of fresh mushrooms does not have any deleterious effects on the quality parameters measured.

The use of PUV irradiation in food processing is mainly implemented as an antimicrobial intervention.[21] The treatment regimen used in this study was examined to determine if the level of irradiation necessary to achieve reasonable levels of vitamin D_2 was effective in reducing microbial loads as well. Neither total aerobic plate count nor yeast and mold levels were reduced with a 3-pulse batch treatment (Table 2). Chikthimmah and Beelman reported that with treatment times varying from 4 to 30 s of PUV, the microbial loads of fresh mushrooms could be reduced by 0.9 to 1.6 log CFU/g.[24] In this present study, a lower dose of PUV irradiation was employed, which could explain why no reduction in microbial load was observed. This could also be due to the use of the B-type lamp, which does not include the most germicidal region of the UV spectrum (i.e., UV-C). Another possible explanation for the lack of a microbicidal effect is that all mushrooms treated in their commercial packages were not fully exposed to PUV radiation: the top layer was likely the only surface that was exposed to PUV radiation, and thus, the microbial population of the lower layer of mushrooms were not affected. Therefore, use of PUV to reduce microbial levels in fresh mushrooms may not be a practical method because the levels of irradiation needed to reduce microbial levels may result in levels of vitamin D_2 that exceed the tolerable upper intake level (UL) of 4000 IU/day.[1]

The use of PUV treatment of fresh mushrooms for the purpose of increasing vitamin D_2 content is an effective and rapid method with no known deleterious effects on product quality. The shortest exposure time of any previous study using continuous UV regimes to achieve a level equivalent to 100% DV/serving was ca. 8 min.[16] In contrast, we have demonstrated in this present study that PUV systems are capable of achieving similar levels of vitamin D_2 with one second or less of total exposure time and without negative effects on appearance. The PUV treatment described in this article does not have an effect on microbial load, which suggests all mushrooms in a commercial package are not exposed to PUV irradiation. Therefore, this study suggests that sliced mushrooms treated in a single layer of small batch system may be the optimal method for enrichment. Furthermore, it was shown that the vitamin D_2 produced under the parameters of this study was stable under commercially relevant storage conditions.

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