Optimizing Vitamin D2 in Mushrooms Report of a Pilot Study to Expose Mushrooms to Ultraviolet Light

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Background

How feasible is it to expose mushrooms to ultraviolet light in order to convert ergosterol to vitamin D2? How much vitamin D2 would optimized mushrooms provide per Nutrition Facts serving (84 g)? This article describes how segments of the mushroom industry, university research departments, national and international agricultural research agencies and the nutrition community collaborated in a pilot study to help answer these questions.

Vitamin D[1] is a rising nutrition star. Long recognized for its role in enhancing calcium absorption necessary for bone health, vitamin D's emerging role in the prevention of chronic diseases including cancer and the regulation of immune response[2] are current topics of research. Very few foods naturally contain Vitamin D – mainly some types of fish – and only a few foods are fortified with vitamin D, such as milk, some juices and cereals.

Cultivated mushrooms unexposed to UV light do not provide vitamin D2, although they are a source of ergosterol, the precursor of vitamin D2[3]. According to literature cited as part of the Food and Drug Administration's proposal (described below), ergosterol has been shown to have anti-tumor and other effects on the immune system and to inhibit the growth of new blood vessels (angiogenesis), important to slow or stop cancer cell growth.

Need for the Pilot Study

The Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA), submitted a proposal to the Mushroom Council entitled: *Optimizing vitamin D2 and ergosterol content of white button and portabella mushrooms: Effects on innate immune response and mammary tumor development in rodents* with Mona S. Calvo, Ph.D., as the Principal Investigator. The proposal focused on exposing mushrooms to short periods of ultraviolet light (UVB), and determining if the enhanced vitamin D2 content could raise vitamin D levels in mice and rats (that is, was bioavailable).

In order to provide the test material for the study diets, the Mushroom Council undertook a pilot study to determine the duration of UV exposure, harvesting and processing techniques needed to maximize the D2 content. The Pennsylvania State University Mushroom Research Center studied the influence of UVB light on mushrooms during the growing and harvesting phase and Monterey Mushrooms studied the influence of UVC light on mushrooms post harvest. Sylvan Inc. conducted preliminary analysis of exposed mushrooms for D2 content. Subsequently, Agrifood Research Finland analyzed D2 in several samples representing different exposure times pre- and post harvest, as well as mushrooms exposed pre-harvest and stored for 3 days at +12C. This

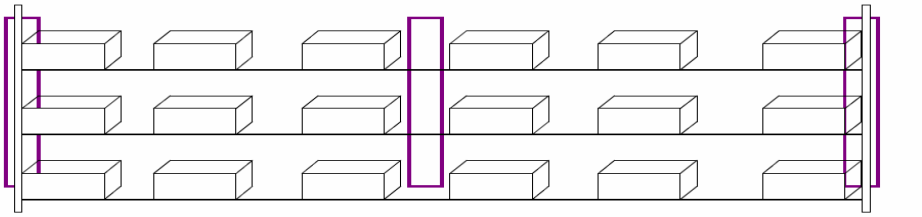
pilot study did not measure change in ergosterol levels in the mushroom samples analyzed.

Exposure to UVB Light

The Mushroom Research Center, Pennsylvania State University, grew white button mushrooms using standard composting, spawning and casing procedures. During the first break harvesting period (17-18 days after casing), mushrooms were exposed to UVB light (wavelengths 290-315 nm) and harvested at 0 (no UV) and 1,2,3,6 and 24 hours. Three UVB light fixtures were hung vertically on each side of the room behind the shelves holding the growing trays (Figure 1). Each fixture held two 48-inch UV bulbs. Mushroom exposure distances ranged from approximately 7 cm to 90 cm away from the light source. Mushrooms were harvested from each tray and randomly sampled for analyses. Mushrooms from the first break were tested for initial color and shelf life and then freeze-dried for analysis of the vitamin D2 content. Additional mushroom samples were stored for 3 days at +12C and then freeze-dried for D2 analysis. This step was done to see if D2 content changed over transportation time and storage conditions which might occur at the retail level.

Figure 1: Mushroom Room Layout for UVB Exposure
Source: Mushroom Research Center, Pennsylvania State University

Each box represents a growing tray. Each side of the room had 3 UV light fixtures (2 48-inch bulbs per fixture) hanging vertically behind the trays (represented by the long rectangles). UV readings were taken directly in front of the front edge of the trays. The lights were approximately 6 inches away from the closest mushrooms in the trays.



Monterey Mushrooms exposed white button and portabella mushrooms post harvest top and gill side exposed for increasing periods of time including an untreated control. Mushrooms were harvested directly from the mushroom growing beds and then exposed within 1 hour of harvest to UVC(190-290 nm) light 15 cm from the light source for 1, 2,5,10 and 15 minutes. Mushrooms were freeze dried for vitamin D2 analysis.

Vitamin D2 Analysis

Sylvan Research analyzed several samples for D2 using HPLC methods reported by Perera[4]. Depending on exposure type and time, D2 values ranged from 5.2 ug/gDW to 8.2 ug/gDW (Table 1) suggesting that mushrooms could be a significant source of vitamin D2.

Table 1: Analysis by Sylvan Research

Sample – All White Button	**ug D2/gmDW**
Control	Not detected
Pre-harvest Exposure	
• 3 hours	5.234

• 6 hours	7.797
• 24 hours	6.305
Post harvest gill side exposed 15 minutes	8.249

To substantiate the preliminary results, Pirjo Mattila, Ph.D, MTT Agrifood Research Finland, whose published methods on D2 analysis are widely referenced,[5] analyzed samples from both Pennsylvania State University and Monterey Mushrooms. Samples were purified by saponification and extraction as well as semipreparative straight-phase HPLC. Vitamin D2 was quantified with reverse-phase HPLC using an internal standard (vitamin D3) method and diode array detection.

Results indicate that a *Nutrition Facts* serving (84 g) of mushrooms exposed to UVB or UVC light could provide vitamin D2 in amounts greater than 100% of the Daily Value (DV) for vitamin D (Table 2. The DV for labeling is 10 µg). Although storage of mushrooms at +12C for three days (which might represent the product at the retail level) resulted in some loss of D2, the amount remaining was still greater than 100% of the Daily Value.

Table 2: Analysis by Mattila, MTT Agrifood Research Finland Calculations of D2 Per Serving and % DV by Feeney

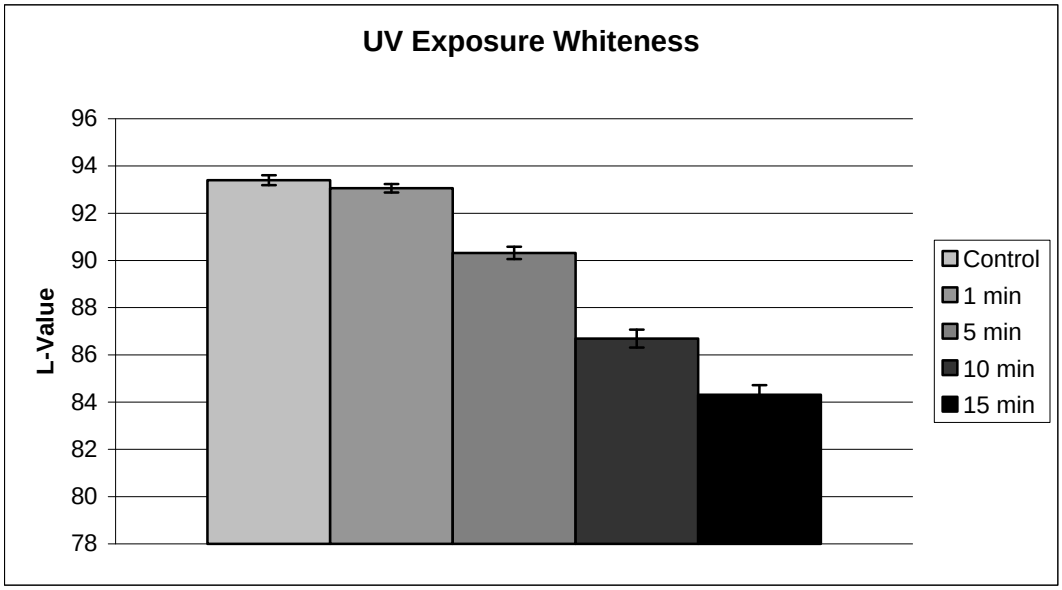
Sample	**µg D2/g DM**	**µg D2/84 g fresh mushroom**	**% DV (10 µg)**
Control			
• White button control	0.06	0.378	3.8%
• Portabella control	0.05	0.315	3.2%
Pre-harvest Exposure UVB White Button Only			
• 1 hour	3.7	23.31	233.1%
• 3 hours	6.2	39.06	390.6%
• 6 hours	8.8	55.44	554.4%
Pre-harvest Exposure UVB Stored 3 days at +12C White Button Only			
• 1 hour	2.3	14.49	144.9
• 3 hours	6.3	39.69	396.9
• 6 hours	6.2	39.06	390.6
Post-harvest Exposure UVC			
• White button top side 5 min	13.8	86.94	869.4%
• White button top side 15 min.	12.9	81.27	812.7%
• Portabella gill side 5 min	7.5	47.25	472.5%
• Portabella gill side 15 min	12.4	78.12	781.2%
Fresh mushrooms = 7.5% DM			

UV Exposure and Discoloration

Discoloration due to UV exposure has been reported[6] and thought to influence acceptability of fresh mushrooms. Monterey Mushrooms tested exposures including an untreated control for 1, 5, 10 and 15 minutes using a chromameter (Konica Minolta BC-

10, Osaka, Japan) by measuring L* and b* values. Figure 2 shows the decreasing brightness with increasing exposure.

Figure 2: UV Exposure Brightness Variability
Source: Monterey Mushrooms



NOTE: L* is a brightness variable and extends from 0 (black) to 100 (white).

Photographs by Pennsylvania State University of UVB treated mushrooms after 3 days of storage at 12 degrees centigrade indicate that although slight browning occurred, the visual difference does not appear great between the control sample and mushrooms exposed for one hour (Figure 3).

Figure 3: Photographs of UVB Treated Mushrooms
Source: Pennsylvania State University

Implications

Results of the pilot study indicate that mushrooms exposed to UVB or UVC light produce vitamin D2 in amounts greater than 100% Daily Value, making mushrooms unique among plant food sources for this vitamin. Market testing is needed to determine whether consumers would find the browning unattractive when informed of the nutrient value of mushrooms exposed to UV light or whether mushrooms optimized for vitamin D would be a specialty product or limited to criminis and portabellas where the browning might not be that noticeable.

Research continues at Monterey Mushrooms and Pennsylvania State University to investigate the optimum time and or wavelength of UV light that can increase vitamin D2 content without negatively influencing nutritive value, fresh yield or quality. In addition, the Mushroom Council plans to study the effects of vitamin D2 optimized mushrooms on immune response and mammary tumors in rodents as described above in the FDA/Calvo proposal.

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[1] Vitamin D (Calciferol) comprises a group of fat soluble sterols. Vitamin D2 or ergocalciferol originates from the plant/fungal sterol ergosterol; D3 or cholecalciferol originates from a precursor of cholesterol when synthesized in the skin by the action of sunlight. National Academies Press. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride* (1997). www.nap.edu.

[2] Vitamin D and Health in the 21st Century: Bone and Beyond. Proceedings of a Conference. DJ Raiten, Guest Editor. *American Journal of Clinical Nutrition Supplement* (2004); 80; 6(S).

[3] Mattila, PH. et al. Sterol and Vitamin D2 Contents in Some Wild and Cultivated Mushrooms. (2002) *Food Chemistry* 76: 293-298.

[4] Perera CO et al. The Effect of Moisture Content on the Conversion of Ergosterol to Vitamin D in Shiitake Mushrooms. (2003). *Drying Technology*; 21: 1091-99.

[5] Mattila PH et al. Vitamin D Contents in Edible Mushrooms. (1994) *Journal of Agricultural and Food Chemistry.* 42:2449-2453.

[6] Mau JL et al. Ultraviolet Irradiation Increased Vitamin D2 Content in Edible Mushrooms. *Journal of Agricultural and Food Chemistry.* (1998) 46: 5269-5272.