Ultraviolet Irradiation Increased Vitamin D 2 Content in Edible Mushrooms

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · DECEMBER 1998

Impact Factor: 2.91 · DOI: 10.1021/jf980602q

3 AUTHORS, INCLUDING:

Jeng-Leun Mau
NCHU

145 PUBLICATIONS **4,485** CITATIONS

SEE PROFILE

Ultraviolet Irradiation Increased Vitamin D_2 Content in Edible Mushrooms

Jeng-Leun Mau,*,† Pei-Ru Chen,† and Joan-Hwa Yang‡

Department of Food Science, National Chung-Hsing University, Taichung 402, Taiwan, Republic of China, and Department of Food and Nutrition, Shih Chien University, Taipei 104, Taiwan, Republic of China

Fresh common (*Agaricus bisporus*) and high-temperature mushrooms (*A. bitorquis*) were irradiated with ultraviolet-C (UV-C) for 0, 0.5, 1, and 2 h at 12 °C. Fresh common, shiitake (*Lentinula edodes*), and straw mushrooms (*Volvariella volvacea*) were irradiated with UV-B for 0, 0.5, 1, and 2 h at 12 °C. After UV-C irradiation for 2 h, vitamin D_2 contents in common and high-temperature mushrooms increased from 2.20 and 4.01 μg/g of dry weight to 7.30 and 5.32 μg/g, respectively. After UV-B irradiation for 2 h, the vitamin D_2 content in common mushrooms reached 12.48 μg/g. UV-B irradiation resulted in higher vitamin D_2 conversion for common mushrooms. After UV-B irradiation for 2 h, vitamin D_2 contents in shiitake and straw mushrooms increased from 2.16 and 3.86 μg/g to 6.58 and 7.58 μg/g, respectively. The increase rates in shiitake and straw mushrooms were not as high as in common mushrooms.

Keywords: *Mushrooms; Agaricus bisporus; Agaricus bitorquis; Lentinula edodes; Volvariella volvacea; ultraviolet-B; ultraviolet-C; vitamin D_2*

INTRODUCTION

Vitamin D is important in human nutrition as a regulator of the metabolism of calcium and phosphate. Vitamin D promotes absorption of calcium and influences the process of bone mineralization. Without vitamin D, mineralization of bone matrix is impaired and collagen synthesis is defective, resulting in rickets in children and osteomalacia in the adults (Combs, 1992). Because most foods are low in vitamin D, several countries fortify frequently consumed foods to prevent rickets. In the United States, recommended dietary allowances (RDAs) for vitamin D are 10 μg for children and 5 μg for adults per day (National Research Council, 1989).

Mushrooms are known to be a good source of vitamin D_2, ergocalciferol. Also, mushrooms contain a high amount of ergosterol, provitamin D_2, which can be converted to vitamin D_2 by ultraviolet (UV) irradiation. UV light consists of three subregions of wavelengths, including UV-C (190−290 nm), UV-B (290−320 nm), and UV-A (320−400 nm). Solar UV irradiation ranges from 290 to 400 nm, and its wavelengths cover both UV-B and UV-A subregions (Jagger, 1985). Because ergosterol is abundant in foods, the best source of vitamin D is sunlight. Therefore, the intake of vitamin D from food is mainly emphasized in both northern and southern latitudes. In addition, the enrichment of vitamin D in foods provides the advantage that calcium in foods can be more available for children, the elderly, and postmenopausal women.

* Address correspondence to this author at the Department of Food Science, National Chung-Hsing University, 250 Kuokuang Rd., Taichung 40227, Taiwan, ROC (telephone 886-4-285-4313; fax 886-4-287-6211; e-mail jlmau@dragon.nchu.edu.tw).

† National Chung-Hsing University.

‡ Shih Chien University.

After UV-B irradiation for up to 2 h, postharvest maturation and microbial counts of common mushrooms were not affected (Chen, 1997). Also, Tai et al. (1998) found that postharvest maturation of common and high-temperature mushrooms was not influenced by UV-C irradiation. The microorganisms in both mushrooms were reduced by only ∼0.5 log (∼68% reduction) as UV-C irradiated for 0.5, 1, and 2 h. However, common mushrooms were obviously tanned after UV-C irradiation for even 0.5 h (Tai et al., 1998) and after UV-B irradiation for 1 h (Chen, 1997). In addition, Chen and Mau (1998) found that volatile compounds of common mushrooms were slightly influenced by UV-C irradiation. In high-temperature mushrooms, the amount of 1-octen-3-ol could be tripled by UV-C irradiation for 1 h.

The vitamin D_2 content in fresh shiitake mushrooms was enriched 2−3-fold by the application of germicidal UV irradiation (Takamura and Hoshino, 1995). Although the induction of vitamin D by UV is already a general idea, information about vitamin D conversion on other mushrooms was not reported. Our objective was to study the effect of different times of UV-C (254 nm) or UV-B (310 nm) irradiation on vitamin D_2 contents of several edible mushrooms.

MATERIALS AND METHODS

Mushrooms. Fresh common [*Agaricus bisporus* (Lange) Imbach], high-temperature [*Agaricus bitorquis* (Quelet) Saccardo], straw [*Volvariella volvacea* (Bull. ex Fr.) Singer], and shiitake mushrooms [*Lentinula edodes* (Berkeley) Pegler] were obtained from Taichung County, Taiwan. Both common and high-temperature mushrooms of uniform size (25−40 mm) and maturity in the button stage (veil intact and tight) were used. Straw mushrooms were harvested at the egg- and bell-shaped stages. Shiitake mushrooms with gills 80% exposed were used.

Packaging and Irradiation. Fresh mushrooms were randomly selected and packaged by placing 100 g in 600-mL PS (polystyrene) trays and overwrapping with MK-PVC film

10.1021/jf980602q CCC: $15.00
Published on Web 11/07/1998

Table 1. Effect of UV-C Irradiation on Contents of Vitamin D_2 and Ergosterol in *A. bisporus* and *A. bitorquis*

mushroom/ compound	content (μg/g of dry mushroom) [relative %][a]			
	0 h[b]	0.5 h	1 h	2 h
A. bisporus				
vitamin D_2	2.20d[c] [100]	4.49c [204]	6.00b [273]	7.30a [332]
ergosterol	273.97a [100]	165.62b [60]	91.47c [33]	33.93d [12]
A. bitorquis				
vitamin D_2	4.01c [100]	4.59b [114]	5.62a [140]	5.32a [133]
ergosterol	50.78c [100]	43.53c [86]	78.57b [155]	254.88a [502]

[a] Based on the control (0 h). [b] Irradiation intensity = 0.2 mW/cm^2. [c] Means with different letters within the same row are significantly different ($p < 0.05$).

[poly(vinyl chloride), 0.016−0.018 mm, Kabaido, Japan] using a B-105 diawrapper (ARC, Japan). After packaging, for either UV-C or UV-B irradiation, three trays of mushrooms were randomly selected as the control; the remaining nine trays were randomly divided into three groups, each for one of the three irradiation treatments (0.5, 1, and 2 h). A tray of mushrooms was placed at 30 cm from the source of irradiation, and the irradiation was performed with a germicidal UV (UV-C) lamp (254 nm, Sankyo Denki, Japan) or UV-B (310 nm, Spectronics Corp., Westbury, NY) for either 0.5, 1, or 2 h at 12 °C.

The UV-C irradiation intensity was measured using a VLX 254 (Vilber Lourmat, France) to be 0.2 mW/cm^2, and the irradiation doses for 0.5, 1, and 2 h were 0.295, 0.606, and 1.471 J/cm^2, respectively. The UV-B irradiation intensity was measured using a VLX 312 radiometer (Vilber Lourmat) to be 0.14 mW/cm^2, and the irradiation doses for 0.5, 1, and 2 h were 0.247, 0.493, and 0.986 J/cm^2, respectively. After UV irradiation, samples were freeze-dried, then ground to powder, and stored in a desiccator before use.

Vitamin D_2 and Ergosterol Assay. Vitamin D_2 and ergosterol were extracted and analyzed according to the method of the AOAC (1990) as modified by Mattila et al. (1994). Freeze-dried mushroom powder (5 g) was mixed with 4 mL of sodium ascorbate (Wako Pure Chemical Co., Osaka, Japan), 50 mL of ethanol (95% pure, Taiwan Tobacco & Wine Monopoly Bureau, Taipei), and 10 mL of 50% potassium hydroxide (Wako), and 100 μg of cholecalciferol (vitamin D_3, Sigma Chemical Co., St. Louis, MO; dissolved in 1 mL of methanol) was added as an internal standard. The mixture was saponified under reflux at 78 °C for 1 h. After cooling, the mixture was first extracted with 15 mL of deionized water and 50 mL of ethyl ether (Alps Chem Co., Hsinchu, Taiwan), then with 10 mL of ethanol and 50 mL of *n*-pentane (Alps), with 50 mL of *n*-pentane, and finally with 20 mL of *n*-pentane.

The organic layers thus obtained were pooled, washed three times with 50 mL of 3% potassium hydroxide in ethanol, and washed with deionized water to neutrality. The organic layer was rotary evaporated to dryness, redissolved in 3 mL of methanol (LC grade, Alps), passed through a filter unit (13 mm, Lida Corp., Kenosha, WI), and filtered using a 0.45 μm NY nonsterile filter (Lida) prior to injection onto a high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Hitachi L-6000 pump, a Rheodyne 7161 injector, a 20 μL sample loop, a Hitachi D-2500 chromatointegrator, a Hitachi L-4000 UV detector, and a Prodigy 5 ODS-2 column (4.6 × 250 mm, 5 μm, Phenomenex Inc., Torrance, CA). The mobile phase was methanol/acetonitrile (LC grade, Tedia Co., Fairfield, OH), 25:75 (v/v), at a flow rate of 1.3 mL/min, and UV detection was at 264 nm. Vitamin D_2 and ergosterol were quantified by comparing the peak area to that of the internal standard.

Statistical Analysis. For either UV-C or UV-B irradiation, three trays from each treatment were examined for each mushroom. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel et al. (1997) to determine the least significant difference among means at the level of 0.05. After multiple comparisons, the means given in the tables were followed with different small letters a−d on the basis of their values and statistical differences. In the case that a mean is followed with ab, this mean was not significantly different from a mean with a and was not significantly different from another mean with b. However, means with different letters were significantly different at the level of 0.05.

RESULTS AND DISCUSSION

Fresh common and high-temperature mushrooms were irradiated with UV-C for 0, 0.5, 1, and 2 h at 12 °C. Vitamin D_2 contents in common mushrooms constantly increased from 2.20 μg/g of dry weight for the control (0 h) to 4.49 μg/g for 0.5 h, 6.00 μg/g for 1 h, and 7.30 μg/g for 2 h, with the increases being 104, 173, and 232%, respectively (Table 1). However, the ergosterol content decreased to 12% for 2 h as the irradiation time became longer. It is obvious that ergosterol was partially converted to vitamin D_2, whereas most of the ergosterol might be UV-degraded. Vitamin D_2 contents in high-temperature mushrooms increased from 4.01 μg/g for the control to 4.59 μg/g for 0.5 h and 5.62 μg/g for 1 h and remained at the level of 5.32 μg/g for 2 h, with the increases being 14, 40, and 33%, respectively. However, ergosterol contents decreased from 50.78 μg/g for the control to 43.53 μg/g for 0.5 h and then inversely increased to 78.57 μg/g for 1 h and to 254.88 μg/g for 2 h.

Without UV-C irradiation, the vitamin D_2 content was higher in high-temperature mushrooms. However, the rate for vitamin D_2 increase as influenced by UV-C irradiation was higher in common mushrooms (2.45 μg/h) than in high-temperature mushrooms (0.67 μg/h). After UV-C irradiation for 2 h, consequently, the vitamin D_2 content was much higher in common mushrooms.

Both mushrooms were discolored as a results of UV-C irradiation. This was in general agreement with the findings of Tai et al. (1998), who found that mushrooms browned along with increased irradiation time. Chen and Mau (1998) found that the amount of 1-octen-3-ol did not increase with the UV-C irradiation applied. However, after UV-C irradiation for 2 h, the amount of 1-octen-3-ol decreased to 61%. On the contrary, 1-octen-3-ol amounts in high-temperature mushrooms increased from 16.72 μg/g of fresh weight for the control to 39.48 μg/g for 0.5 h, 54.65 μg/g for 1 h, and 48.82 μg/g for 2 h (Chen and Mau, 1998). The pattern of 1-octen-3-ol amounts in high-temperature mushrooms as influenced by UV-C irradiation is similar to that of vitamin D_2 contents in the present study.

1-Octen-3-ol was produced in mushrooms as a result of cell damage or subjection to stress, such as temperature and radiation (Mau et al., 1993). The pattern of ergosterol decrease in common mushrooms revealed that UV-C irradiation might activate some enzymatic reactions to degrade ergosterol. On the contrary, the change in ergosterol in high-temperature mushrooms

Table 2. Effect of UV-B Irradiation on Contents of Vitamin D_2 and Ergosterol in *A. bisporus*, *L. edodes*, and *V. volvacea*

mushroom/ compound	content (μg/g of dry mushroom) [relative %][a]			
	0 h[b]	0.5 h	1 h	2 h
A. bisporus				
vitamin D_2	2.20d[c] [100]	5.74c [261]	8.51b [387]	12.48a [567]
ergosterol	273.97a [100]	21.61b [8]	40.39b [15]	56.69b [21]
L. edodes				
vitamin D_2	2.16d [100]	3.71c [172]	4.69b [217]	6.58a [305]
ergosterol	297.09b [100]	316.61b [107]	373.15a [126]	286.16b [96]
V. volvacea				
vitamin D_2	3.86d [100]	4.98c [129]	6.28b [163]	7.58a [196]
ergosterol	185.89b [100]	96.92c [52]	233.56a [126]	215.81a [116]

[a] Based on the control (0 h). [b] Irradiation intensity = 0.14 mW/cm^2. [c] Means with different letters within the same row are significantly different ($p < 0.05$).

suggested that mushrooms might trigger some biochemical pathway for ergosterol synthesis in response to UV irradiation as occurred in 1-octen-3-ol formation. Accordingly, regarding the change in ergosterol content, both mushrooms responded differently to UV-C irradiation.

Fresh common, shiitake, and straw mushrooms were irradiated with UV-B for 0, 0.5, 1, and 2 h at 12 °C. Vitamin D_2 contents in common mushrooms remarkably increased from 2.20 μg/g for the control to 12.48 μg/g for 2 h, with the increase being 467% (Table 2). Compared to UV-C irradiation in Table 1, UV-B irradiation resulted in higher vitamin D_2 conversion (5.04 μg/h) than UV-C irradiation in common mushrooms (2.45 μg/h). The ergosterol content in common mushrooms was greatly affected and significantly reduced by UV-B irradiation for 0.5 h. The loss of ergosterol in common mushrooms by UV-B irradiation was consistent with the results by UV-C irradiation in Table 1. It revealed that both UV-B and UV-C irradiation could increase vitamin D_2 contents in common mushrooms, whereas substantial ergosterol loss was observed. In addition, common mushrooms were discolored as a results of UV-B irradiation. This result was reminiscent of the findings in Chen (1997).

Vitamin D_2 contents in shiitake and straw mushrooms steadily increased from 2.16 and 3.86 μg/g for the control to 6.58 and 7.58 μg/g for 2 h, with the increases being 205 and 96%, respectively (Table 2). Ergosterol contents in shiitake mushrooms were less affected than those in common and high-temperature mushrooms. Ergosterol contents in straw mushrooms decreased from 185.89 μg/g for the control to 96.92 μg/g for 0.5 h and then increased to the slightly higher level of 233.56 μg/g for 1 h and 215.81 μg/g for 2 h. Due to their dark appearance in nature, the surface discoloration of shiitake and straw mushrooms was not noticeable. The change in ergosterol contents was not as significant as that in common or high-temperature mushrooms. However, their dark appearance might be one of the reasons accounting for these stable ergosterol contents in two mushrooms. To understand the change of ergosterol as a result of UV irradiation, further investigation is needed.

Although UV-B irradiation also affected the vitamin D_2 conversion in shiitake and straw mushrooms, the increase rates (2.15 and 1.86 μg/h, respectively) were not as high as in common mushrooms (5.04 μg/h). These results showed that both UV-C and UV-B irradiation could increase the vitamin D_2 content in mushrooms. Also, the increases by UV-C and UV-B irradiation were both time-dependent, that is, dose-dependent. Although the conversion of vitamin D by UV is already a general idea, the results in this study showed that vitamin D conversion in vivo was not as high as expected with regard to the ergosterol content in mushrooms.

Shiitake mushrooms cultivated outdoors contained 5−7-fold higher amounts of vitamin D_2 than those cultivated indoors (Takamura and Hoshino, 1995). These authors also found that, when harvested on sunny days, vitamin D_2 contents of shiitake mushrooms cultivated outdoors were higher than those harvested on cloudy days. After solar irradiation (both UV-B and UV-A), vitamin D_2 contents were 6-fold higher in fresh than in dried shiitake mushrooms. In addition, vitamin D_2 contents in fresh shiitake mushrooms could be enriched 2−3-fold by the application of germicidal UV (254 nm) irradiation at 0.117 mW/cm^2 for 1 min.

Takamura and Hoshino (1995) reported that vitamin D_2 contents in fresh shiitake mushrooms ranged from 0.37 to 1.22 μg/g of dry weight, lower than that of shiitake mushrooms, 2.16 μg/g in Table 2. The difference in vitamin D_2 contents might be partially due to differences in strains and cultivation conditions. However, less UV light at higher latitudes of Japan (30−45° N) might be the major reason accounting for the lower vitamin D_2 contents as compared to the latitudes of Taiwan (22−25° N).

The addition of calcium chloride to irrigation water could improve the quality and shelf life of common mushrooms (Beelman et al., 1992). Miklus (1993) indicated that calcium contents in common mushrooms irrigated with 0.3% calcium chloride were approximately doubled to nutritionally significant levels (100−200 μg/g of dry weight). In this study, vitamin D_2 contents in common mushrooms could be remarkably enriched by UV-B irradiation. Because vitamin D_2 is essential for calcium absorption in the small intestine, the combination of calcium chloride irrigation treatments during fruiting and UV-B irradiation after harvest would be of great interest to improve the nutraceutical value of common mushrooms. However, the surface discoloration resulting from UV irradiation might influence the acceptability of the product in the fresh market.

ACKNOWLEDGMENT

We thank Mr. Chih-Yuan Chang for providing common, high-temperature, and straw mushrooms and Mr. Hsi-Ming Chen for shiitake mushrooms.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.

Beelman, R. B.; Bartley, C. E.; Mau, J.-L.; Miklus, M. B.; Solomon, J. M.; Royse, D. J.; Winnet, J. R.; Muthersbaugh,

H. R. Irrigation water treatments to improve quality and shelf life of *Agaricus* mushrooms. *Mushroom News* **1992**, *40* (2), 18−23.

Chen, P.-R. Effect of ultraviolet irradiation on quality of common mushrooms and other edible mushrooms. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan, 1997.

Chen, P.-R.; Mau, J.-L. Effect of ultraviolet-C irradiation on volatile compounds of common and high-temperature mushrooms. *Food Sci. (ROC)* **1998**, *25*, 497−504.

Combs, Jr., G. F. *The Vitamins: Fundamental Aspects in Nutrition and Health*; Academic Press: San Diego, CA, 1992.

Jagger, J. *Solar-UV Actions on Living Cells*; Praeger: New York, 1985.

Mattila, P. H.; Piironen, V. I.; Uusi-Rauva, E. J.; Koivistoinen, P. E. Vitamin D contents in edible mushrooms. *J. Agric. Food Chem*. **1994**, *42*, 2449−2453.

Mau, J.-L.; Beelman, R. B.; Ziegler, G. R. Factors affecting 1-octen-3-ol in mushrooms at harvest and during postharvest storage. *J. Food Sci*. **1993**, *58*, 331−334.

Miklus, M. B. Studies on the role of calcium added to irrigation water on quality of fresh mushrooms (*Agaricus bisporus*). Master's Thesis, Pennsylvania State University, University Park, PA, 1993.

National Research Council. *Recommended Dietary Allowances*, 10th ed.; National Academy Press: Washington, DC, 1989.

Steel, R. G.; Torrie, J. H.; Dickey, D. A. *Principles and Procedures of Statistics: A Biometrical Approach*; McGraw-Hill: Singapore, 1997.

Tai, H.-Y.; Chen, P.-R.; Mau, J.-L. Effect of ultraviolet-C irradiation on stored quality of *Agaricus bisporus* and *A. bitorquis* mushrooms. *Food Sci. (ROC)* **1998**, *25*, 94−103.

Takamura, K.; Hoshino, H. Vitamin D_2 of shiitake mushrooms. *New Food Ind*. **1995**, *37* (8), 33−39.

Received for review June 4, 1998. Revised manuscript received September 15, 1998. Accepted September 18, 1998. The study was supported in part by the National Science Council, Project NSC86-2815-C-005-056-B.

JF980602Q