JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Vitamin D Mushrooms: Comparison of the Composition of Button Mushrooms (*Agaricus bisporus*) Treated Postharvest with UVB Light or Sunlight

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ABSTRACT: This study compared the compositional changes in mushrooms exposed to sunlight with those occurring after commercial ultraviolet (UV) light processing. Button mushrooms (75 kg) were processed in the presence or absence of UVB light; a third group was exposed to direct sunlight. Mushroom composition was evaluated using chemical analyses. Vitamin D concentrations were 5, 410, and 374 μg/100 g (dw) in control, UVB, and sunlight groups, respectively. On a dry weight basis, no significant changes in vitamin C, folate, vitamins B_6, vitamin B_5, riboflavin, niacin, amino acids, fatty acids, ergosterol, or agaritine were observed following UVB processing. Sunlight exposure resulted in a 26% loss of riboflavin, evidence of folate oxidation, and unexplained increases in ergosterol (9.5%). It was concluded that compositional effects of UVB light are limited to changes in vitamin D and show no detrimental changes relative to natural sunlight exposure and, therefore, provide important information relevant to the suitability and safety of UVB light technology for vitamin D enhanced mushrooms.

KEYWORDS: mushroom, *Agaricus*, ultraviolet light, sunlight, UVB, UVC, vitamin D, ergocalciferol, ergosterol, agaritine, composition, fatty acid, amino acid, folate, vitamin C, vitamin B_6, riboflavin, niacin, pantothenic acid

INTRODUCTION

New dietary reference intakes for vitamin D have been published by the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) with daily allowances of 600 IU (15 μg) recommended for children and adults and higher intakes of up to 800 IU (20 μg) suggested for individuals >70 years of age.[1] Achieving the Recommended Daily Allowance (RDA) of vitamin D through the consumption of a well-balanced diet alone can be difficult because there are few natural food sources of vitamin D; limitations on food fortification imposed by strict regulatory policies further restrict the availability of vitamin D containing foods in the marketplace. Thus, there is a need to both introduce new vitamin D containing foods to the marketplace and promote increased consumption of existing and/or underutilized natural sources of vitamin D such as salmon and other types of fish.

Mushrooms are an unappreciated food source of vitamin D, and various species of commercially available wild mushroom have been shown to be excellent sources of vitamin D;[2] in some instances the concentrations of vitamin D (vitamin D_2; ergocalciferol) rival those of vitamin D (vitamin D_3; cholecalciferol) present in oily fish. For example, concentrations of vitamin D_2 were reported to range between 10.7 and 29.8 μg/100 g serving (428−1192 IU per serving) in wild-harvested chanterelle mushrooms,[3−5] up to 58.7 μg/100 g serving (2348 IU/100 g serving) in wild porcini mushrooms, and up to 40 μg/100 g serving (1600 IU/100 g serving) in sun-dried shitake mushrooms.[5,6] The high level of vitamin D in wild mushrooms compared to their cultivated comparators is attributed to their growth outdoors, which results in exposure of the mushroom to sunlight. The ultraviolet (UV) radiation from sunlight catalyzes a unique photochemical reaction whereby the fungal sterol, ergosterol, is converted to vitamin D_2 through a series of photochemical and thermal reactions; this photochemical process is very similar to the process by which vitamin D_3 is produced in human skin.[7]

Commercial mushroom growers have recently incorporated sources of UV light into their production processes to enhance the vitamin D content of mushrooms by simulating the production of vitamin D that occurs in mushrooms exposed to sunlight in their natural environments. Recent analyses conducted on vitamin D enhanced mushrooms sampled from the U.S. marketplace show that current products available contained between 3 and 20 μg (120−800 IU) of vitamin D per 100 g serving.[2] Although numerous research studies characterizing the production of vitamin D in mushrooms exposed to various types of UV light have been published in the literature,[8−11] there are no studies that have specifically evaluated mushrooms produced using commercial processing conditions. Information within the literature on the effects of UV light on the nutrient and sterol composition of mushrooms are limited.

Thus, the primary objectives of this investigation are threefold: to investigate the suitability of commercial-scale UV light processing techniques for the production mushrooms with consistent and reliable concentrations of vitamin D; to assess the effect of commercial UV light processing on the nutritional

Received: March 29, 2011
Revised: July 6, 2011
Accepted: July 8, 2011
Published: July 08, 2011

Table 1. Experimental Study Design Detailing the Distribution of *Agaricus bisporus* Mushroom Crops Used in the Study

			A. bisporus processed in the presence of light	
time	batch/crop no.	*A. bisporus* (control)	UVB	sunlight
day 1	193A184W	5 kg (18 bags[a]) C1[b]	5 kg (18 bags) UV1[b]	5 kg (18 bags) S1[b]
day 2	193A184AW	5 kg (18 bags) C2	5 kg (18 bags) UV2	5 kg (18 bags) S2
	178A176W	5 kg (18 bags) C3	5 kg (18 bags) UV3	5 kg (18 bags) S3
day 3	908038	5 kg (18 bags) C4	5 kg (18 bags) UV4	5 kg (18 bags) S4
	908041	5 kg (18 bags) C5	5 kg (18 bags) UV5	5 kg (18 bags) S5

[a] 10 oz (283 g) per bag. [b] C1−C5, UV1−UV5, and S1−S5 represent sample codes for replicate batches. Mushroom treatments were conducted over 3 days. To account for potential variability due to crop location and mushroom maturity, each batch of mushrooms obtained from a particular location within the facility, as designated by the above crop no., was randomly distributed to each of the three treatment groups.

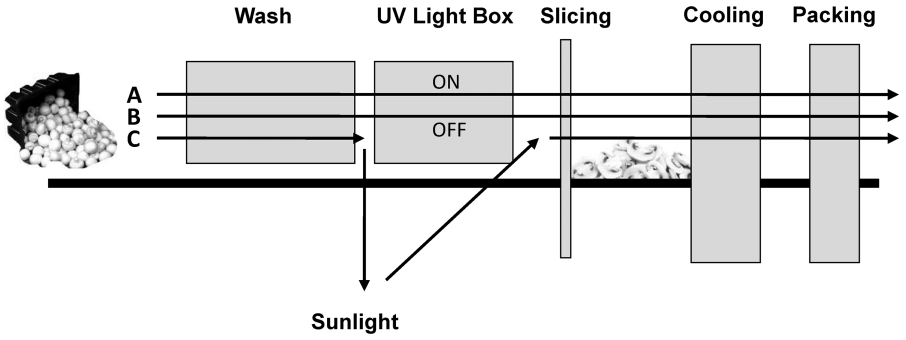

Figure 1. Schematic overview of mushroom processing. Mushrooms were processed as five separate 15 kg batches, with each 15 kg batch randomized to one of three treatment groups: (A) UVB exposure (UV light box on); (B) control (UV light box off); (C) sunlight exposure (UVB light box off). For the sun exposure treatment, mushrooms were removed from the processing conveyor and transported to a designated sunlight treatment area for 2.5 h of sunlight exposure. Mushrooms were then rebagged and returned to the processing facility for slicing and bagging.

composition of vitamin D mushrooms intended for introduction to the marketplace; and to compare the compositional changes occurring in mushrooms exposed to sunlight with those occurring in mushrooms exposed to UVB light.

MATERIALS AND METHODS

Mushroom Samples and Treatment Conditions. White button mushrooms (*Agaricus bisporus*, delta strain) were obtained from Monterey Mushrooms, Inc., Royal Oaks facility (Watsonville, CA) on three separate days between August 31 and September 8, 2009. Mushrooms used for the experiment were medium sized, with cap diameters typically ranging between 3 and 4.5 cm as designated by the facility, and each batch of mushrooms was harvested in the morning by trained individuals. Following harvesting, the mushrooms were stored in plastic storage containers and transferred to the mushroom processing facility, which is maintained at a temperature of approximately 10 °C throughout the day.

Agricultural foods are inherently variable in their nutrient composition, which is influenced by a number of factors such as geographical location, soil composition, season, plant maturity, and cultivar/strain type. To ensure that between treatment group differences in mushroom composition would not arise due to external factors such as crop location within the facility, mushroom maturity, and selection bias during harvesting, each batch of harvested mushrooms (>15 kg) was equally distributed among all of the treatment groups [control (nonexposed), UVB, and sunlight]. A sunlight comparator group was included in the experiment to determine if UVB exposure of mushrooms imparted chemical changes to the mushroom nutrient composition that are unique or not observed in wild mushrooms exposed to sunlight. In total >75 kg of mushrooms were used for this experiment, consisting of mushrooms obtained from five different crops. The sampling plan used in the experiment is shown in Table 1, and each treatment group consisted of five 5 kg subgroups. Each subgroup was produced from 18 10 oz bags (283 g) of processed ready-to-eat mushrooms.

All conditions employed during processing of UVB exposed and nonexposed controls were representative of conditions used during production of Monterey's commercial products (10 oz bag, Clean N Ready Bagged Packs). Exceptions were made for the sun-exposed mushrooms, which are not a commercial product and required transport to a suitable outdoor area for exposure to sunlight. The dose of UV light was monitored using a 4.5 in. diameter × 0.5 in. thick electro-optic UV Power Puck (standard model; EIT Instruments, Sterling VA). The instrument was used to verify dosing prior to mushroom processing and during mushroom processing by placing the unit on the conveyer with the mushrooms, ensuring UV exposure was monitored on a real-time basis. Total UV energy density in joules/cm^2, for UVA, UVB, UVC, and UV visible (UVV) wavelengths, was obtained during each pass. UV processing conditions were targeted to achieve a UVB dose of 1.08 J/cm^2.

A schematic overview of the processing conditions is presented in Figure 1. Briefly, whole mushrooms from each batch were dumped onto the processing conveyer without regard for orientation. The mushrooms then proceeded through a washing stage, and those to receive the commercial UVB treatment passed under a custom UV exposure unit housing several mercury vapor UVB emitting bulbs. The bulbs were approximately 10−15 cm from the mushrooms and operated on a continual basis, delivering a target UVB dose of 1.08 J/cm^2. The UV power puck was placed on the conveyer on a continual basis until all mushrooms in the treatment group cleared the UV lamps, and after each

pass the UV reading was recorded. The conveyer speed permitted sufficient time to obtain UV readings over 2 to 3 passes during mushroom exposure. Following UV exposure, mushrooms were then sliced to the standard thickness of Monterey's commercial sliced product (0.635 cm) and then continued along the conveyer through several cooling chambers to weigh scales, followed by a final bagging stage. The weight of each bag was verified in-line using electronic weigh scales, and bags outside the 10 oz (283 g) weight were rejected.

The control mushrooms were processed immediately after the UV-exposed mushrooms in an identical manner; however, UV lamps were turned off. Care was taken to ensure that any residual UV-exposed mushrooms were removed from the processing line, and for further assurance that control samples were not contaminated with UV treated mushrooms, the first four bags of control mushrooms clearing the assembly line were discarded. Mushrooms intended for sun exposure were processed as the control and UV groups; however, mushrooms were collected by hand after the wash stage and packed into a single large black plastic bag for transport to the sun exposure area.

For the sun exposure treatment, the washed mushrooms were placed on a large wax tarp on a concrete pad and were spread randomly to form a single layer of whole mushrooms. Temperature sensing probes were inserted into two mushrooms to monitor the internal mushroom temperature and external ambient temperature over the 2.5 h sun-exposure period. This exposure duration was determined through the use of pilot studies, with the intent to produce comparable quantities of vitamin D during sunlight exposure relative to those targeted in the commercial vitamin D mushroom products. To achieve consistent sunlight irradiation, sun exposure occurred at approximately the same time targeting the peak ultraviolet exposure of the mid-day sun (between 11:30 a.m. and 12:45 pm). Weather conditions in Wattsonville, CA, were sunny and cloudless on each day, and monitoring of UV light dosing was conducted using the same UV power puck described above. To ensure that the sun-exposed mushrooms were not further exposed to UV light following the 2.5 h exposure period, the mushrooms were carefully placed back into a black plastic bag, returned to the processing facility, and deposited on the conveyer line for slicing and bagging.

Once a completed treatment group of mushrooms was bagged and labeled, each bag was immediately frozen at −30 °C, placed as a single layer in one of two large chest freezers to achieve rapid freezing. The next day, the frozen sample groups were packed in a large Styrofoam cooler for shipping. The cooler contained a layer of dry ice on the bottom, covered with wax paper. Eighteen bags of frozen mushrooms were then placed on the wax paper, covered with a second layer of wax paper, and then covered with dry ice. Treatment groups were not mixed, and each cooler contained 18 bags from a single treatment. Coolers were labeled and shipped via overnight express delivery to the Food Analysis Laboratory Control Center at Virginia Tech (VT) (Blacksburg, VA) for compositing and compositional analyses.

Compositing. Homogenized composites of each 5 kg (18 bags) treatment sample were prepared. All stages of handling and compositing were conducted under UV protection. The mushrooms were received frozen and were maintained frozen throughout homogenization. Upon arrival, the frozen mushrooms from a single treatment sample (18, 10 oz bags) were homogenized in liquid nitrogen using a 29 L industrial food processor (Robot Coupe USA, Jackson, MS), resulting in a uniformly ground composite. Subsamples of each composite to be used for nutrient analyses were dispensed, while being maintained frozen with liquid nitrogen and with minimal headspace, into glass jars with Teflon-lined lids [except 7 oz Whirl-Pak polyethylene bags (Nasco, Ft. Atkinson, WI) containing ∼25 g were used for subsamples to be assayed for agaritine] and sealed under nitrogen gas. Each sample container was labeled using automated software tracking system with a unique identification number that did not identify the sample treatment and was traceable within the data system to the original sample identification number.

Compositional Analyses. *Analytical Methods.* Analyses of moisture, folate, and ergosterol were conducted at Virginia Tech. Proximates, fatty acids, vitamin B_6, riboflavin, and pantothenic acid were analyzed at Silliker, Inc. (Chicago Heights, IL); amino acid analysis was performed at Eurofins Scientific (Des Moines, IA); niacin and vitamin C determinations were performed by Covance (Madison, WI); vitamin D was analyzed at Heartland Assays (Ames, IA); and agaritine was assayed by Sylvan, Inc. (Kittanning, PA). All analyses were conducted using established standard methods (e.g., AOAC International) and/or published and validated methodology. Brief descriptions of the methods are given below.

Samples for each component to be analyzed were batched with the blind food matrix control samples and shipped to the designated laboratories frozen, on dry ice, via express overnight delivery. Analyses were conducted in singlicate; and blinded control composite samples were included in each assay to provide an estimate of analytical uncertainty for individual composites assayed in singlicate.

Proximates. The moisture in each composite was determined by vacuum-drying to constant weight at 65−70 °C and 635 mmHg using AOAC 926.08.[15] Ash analysis was conducted using AOAC 935.39.[15] Total nitrogen was determined using the Kjeldhal method using AOAC 991.20E,[15] with protein calculated as N × 6.25. Analysis of total fat was conducted using Mojo acid hydrolysis in accordance with AOAC 933.05,[15] and total carbohydrates were determined from the fresh weight derived data by difference calculation using the following formula: % carbohydrate = 100% − (% protein + % fat + % ash + % moisture).[16]

Amino Acids. Amino acids were analyzed using a modified AOAC 982.30[15] reference method consisting of acid hydrolysis in 6 N HCl at 110 °C for 24 h and quantitation occurring via ion exchange chromatography with a postcolumn ninhydrin reaction and UV−vis detection. Tryptophan was analyzed as described under AOAC 988.15[15] and consisted of alkaline digestion with lithium hydroxide (110 °C) for 22 h followed by quantitation via reversed-phase chromatography with UV−vis detection. Cysteine and methionine were determined using AOAC 994.12,[15] with modifications, with quantitation conducted using ion exchange chromatography with *o*-phthalaldehyde (OPA) postcolumn reaction.

Fatty Acids. Fat by fatty acid profile was analyzed by gas chromatography in accordance with AOAC 969.33.[15]

Vitamin D. Vitamin D_2 was analyzed using high-performance liquid chromatography (HPLC)-UV detection after saponifcation (methanolic KOH) and three times extraction of the composites with hexane/ethyl acetate followed by purification using sequential column chromatography prior to quantitation of vitamin D_2 by HPLC with UV detection as previously described and validated.[2,17] Method validation for vitamin D_2 included recovery of 60 ng of vitamin D_2 plus [^{3}H]-vitamin D_3 internal standard added to 10 samples of the mushroom control composite described below containing a similar amount (∼60 ng) of vitamin D_2 in the analytical portion. Additionally, vitamin D_2 was confirmed by analysis of the mushroom control composite, both at the USDA Food Composition and Methods Development Laboratory (FCMDL) using ultraviolet mass spectrometric analysis previously reported[2] and also independently by an experienced commercial laboratory.

Vitamin C. Vitamin C was quantified as total ascorbic acid by fluorescence detection using AOAC 967.22, modified.[15] Briefly, vitamin C in the sample was extracted, oxidized, and reacted with *o*-phenylenediamine to produce a fluorescent compound and then quantified by comparing the sample extract fluorescence to the fluorescence of known standards.

Vitamin B_6. Vitamin B_6 was quantitated following USFDA/AOAC 400 methodology.

Niacin. Niacin was analyzed using standard microbiological methodology: AOAC 944.13, modified; AOAC 960.46, modified; AOAC 985.3, modified;[15] United States Pharmacopeia (USP), Vol. 23, pp 1743−1745;[18]

methods of analyses for infant formulas, Infant Formula Council, modified.[19] Briefly, samples were hydrolyzed with sulfuric acid, and following pH adjustment the amount of niacin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus plantarum*, with the growth response of a known niacin standard via turbimetric measurement.

Riboflavin. Measurements of riboflavin concentration were determined using AOAC 970.65.[15]

Folate. Folate was extracted using a previously validated and reported method,[20,21] involving trienzyme extraction and liquid chromatography—mass spectrometry for quantitation. The major vitamers, 5-methyltetrahyrofolate (5-CH_3-H_4 folate), 5-formyltetrahydrofolate (5HCO-H_4 folate), and 10-formyl folate (10-HCO folate), were quantified, with a limit of detection of 1 μg/100 g for each component.

Ergosterol. Ergosterol was quantified in the alkaline saponified total lipid extracts by capillary gas chromatography of the trimethylsilyl ether derivative as previously reported by Phillips et al.[22]

Agaritine. One gram of the mushroom composite was mixed with 70 mL of HPLC grade methanol (containing 1% $NaHSO_3$ preservative) in an Erlennmyer flask for 60 min under an aluminum foil cover to prevent exposure to UV light. The mixture was then vacuum filtered (Whatman no. 1 filter) and the flask rinsed with methanol to produce a final volume of 100 mL in a graduated cylinder. Fifty milliliters of the extract was then transferred to a round-bottom flask and evaporated to dryness (Buchi Rotavapor R-205). The dried extract was resuspended in 5.0 mL of mobile phase buffer (0.005 N NaH_2PO_4, pH 4.25) and sonicated for 10 min. Solid phase extraction cleanup was conducted using a Bond Elut C18 cartridge (500 mg/3 mL Varian) placed on a vacuum manifold and conditioned with 3 × 2.5 mL methanol followed by 3 × 2.5 mL of mobile phase buffer. Four milliliters of the sample solution was then added to the cartridge and rinsed with 1 mL of mobile phase buffer. The eluate was collected in a 15 mL centrifuge tube and filtered through a 0.45 μM Millipore filter into an HPLC vial. A volume of 10 μL of the filtered sample was injected into a Hitachi 7000 with an autosampler, a column heater, and a UV—vis detector and eluted through a reverse phase C18 column (Purospher PR-18 column, Merck; 250 mm × 4 mm, 5 μm) using 0.005 N NaH_2PO_4, pH 4.25, as the mobile phase at a flow rate of 0.8 mL/min, and a temperature of 31.5 °C. The UV detection of the eluate was performed by UV spectral analysis (Spectronic Genesys 2; molar absorbance of 12,000 at UV max of 237 nm).

Quality Control. A blinded food matrix control sample was included in each analytical batch for each component analyzed. Coefficients of variance (CV) for each nutrient in these control samples have been prepared using established protocols and previously analyzed as part of other research projects including the U.S. Department of Agriculture National Nutrient Analysis Program,[23] and analytical results for these samples served as a quality control check for assurance that all data generated for the mushroom composites could be considered reliable. The following control composites were used: for vitamin D, vitamin C, and ergosterol, a composite consisting of 50% vitamin D mushrooms and 50% standard white button mushrooms;[2] for amino acids, fatty acids, proximates, riboflavin, and pantothenic acid, a slurried mixed vegetable composite. For niacin and vitamin B_6 the following commercial certified reference materials were used, respectively: NIST SRM2385 Slurried Spinach (National Institute of Standards and Technology, Gaithersburg, MD) and BCR CRM 485 Lyophilized Mixed Vegetables (Institute of Reference Materials and Methods, Geel, Belgium, purchased from RT Corp., Laramie, WY). No control composite or reference material was available for agaritine; therefore, one mushroom sample composite was randomly selected (Composite UV4; UVB treatment), and blinded triplicate subsamples were included in the analytical batch to obtain an estimate of the CV for the method for the mushroom composite matrix.

Statistics. The concentration of each analyte is presented as the mean (n = 5) followed by standard deviations in parentheses. Because the goal was to compare changes in composition other than moisture, component concentrations are reported and were compared on a dry weight basis. Statistical analyses were performed using GraphPad InStat version 3.06 for Windows (GraphPad Software, San Diego, CA), as follows. For measures passing the Kolmogorov and Smirnov test for normality, statistical comparison of the treatment groups was conducted using a one-way analysis of variance, and the differences between means were compared using Tukey's HSD method. For measures failing the normality test, a nonparametric test was used (Kruskal—Wallis), and post-test analyses of significant differences were conducted using Dunn's test. Statistical significance was assigned at $p < 0.05$ for all analyses.

RESULTS AND DISCUSSION

In this study, the composition of *A. bisporus* mushrooms treated postharvest with UVB light was compared to mushrooms exposed to sunlight. The sunlight comparator treatment was selected as a controlled surrogate for assessment of changes occurring in wild mushrooms, which are naturally exposed to sunlight and which have a long history of safe consumption. Mushrooms processed in the absence of UV light were used as a representative baseline comparator for each of the treatments.

For selection of the nutrients analyzed in this study, it was recognized that foods contain a vast number of constituents and it is impossible, logistically and financially, to comprehensively analyze every component in a given food type. Ideally, an analytical plan should characterize the specific food components of a given crop or food type that are believed to be important to human nutrition and safety. The specific nutrients and bioactive components analyzed should be selected with the aim of efficiently characterizing the food in a manner that is reasonable given the financial costs of analytical testing. As reported by Greenfield and Southgate,[12] a compositional sampling strategy should select nutrients and other food components based on the following considerations: the basic need for information; health problems in the country concerned; the state of current thinking in the nutritional and toxicological sciences; the availability of existing data; the existence of adequate analytical methods; the feasibility of analytical work; national and international nutrition labeling regulations.

The Organisation for Economic Co-operation and Development (OECD) task force for the Safety of Novel Foods and Feeds has produced a number of science-based consensus documents, which detail the compositional analytes that should be assessed when new varieties of common agricultural food commodities are characterized.[13] These standards were developed using an approach similar to that defined by Greenfield and Southgate,[12] above, and are considered mutually acceptable among its member countries. Although these documents were originally developed in support of regulatory assessments of genetically modified foods or feeds, they are well suited to general use in substantial equivalence determinations of foods produced using new technologies. A consensus document on the compositional considerations for new varieties of *A. bisporus* has been published by the OECD task force.[13] On the basis of considerations provided in this document, and those detailed by Greenfield and Southgate as described above, the following key nutritional and non-nutritional analytes were selected for analysis: proximates (moisture, ash, nitrogen, carbohydrate, fat), vitamins (vitamin D_2, vitamin C, vitamin B_6, riboflavin, niacin, pantothenic

Table 2. Proximate Composition (Percent Dry Weight) of *Agaricus bisporus* Mushrooms Processed with UV Light[a]

		A. bisporus processed in the presence of light	
parameter	*A. bisporus* (control)	UVB	sunlight
moisture	92.7 (0.3)	92.7 (0.4)	92.0 (0.3)[b]
ash	9.80 (0.62)	9.97 (0.63)	10.04 (0.57)
protein	33.31 (2.23)	34.78 (0.99)	34.70 (1.66)
carbohydrate	38.14 (2.83)	36.32 (4.11)	32.65 (5.04)
fat	3.75 (0.37)	4.69 (0.81)	4.36 (0.73)

[a] Data are expressed as the mean ($n = 5$) followed by standard deviation in parentheses. [b] $p < 0.05$ vs control. Otherwise, means within each row did not differ significantly ($p > 0.05$).

Table 3. Vitamin D_2 and Ergosterol (Provitamin D_2) Content (per 100 g) of *Agaricus bisporus* Mushrooms Processed with UV Light[a]

		A. bisporus processed in the presence of light	
parameter	*A. bisporus* (control)	UVB	sun
ergosterol (provitamin D_2) (mg dry weight)	578.2 (29.8)	579.5 (20.7)	633.4 (17.4)[b]
vitamin D_2 (μg dry weight)	5.5 (4.6)	410.9 (56.7)[c]	374.5 (86.6)[c]
IU per 84 g serving (fresh weight)	14 (11)	1008 (139)	918 (212)
% of RDA[d]	2	168	153

[a] Data are expressed as the mean ($n = 5$) followed by standard deviation in parentheses. [b] $p < 0.01$ vs control. [c] $p < 0.001$ vs control. Otherwise, means within each row did not differ significantly ($p > 0.05$). [d] RDA, Recommended Daily Allowance, 15 μg (600 IU) for individuals aged 1−70.[1]

acid, and folates), macronutrients (fatty acids and amino acids), and sterols (ergosterol). *A. bisporus* mushrooms contain small quantities of the naturally occurring phenylhydrazine alkaloid, agaritine. Although historically agaritine has been viewed as a natural toxin of the white button mushroom, safety data available in the literature are conflicting, and no validated toxicology studies conducted with agaritine are available.[14] Nevertheless, because agaritine has historically been viewed as an undesirable compound, and it is included in the OECD consensus document on the compositional considerations for new varieties of *A. bisporus*, analytical data for agaritine also were obtained.

Quality Control. With the exception of niacin, all control composite results were within the 95% confidence intervals previously established (data not shown). The assayed concentration of 0.75/mg 100 g dry weight (dw) for vitamin B_6 was within the certified limits for BCR CRM 485 (0.40−0.56 mg/100 g dw). The assayed niacin concentration in NIST SRM 2385 (0.75 mg/100 g) was nearly 2-fold above the upper limit of the reference range of 0.25−0.34 mg/100 g; however, although this result may reflect the accuracy of the absolute niacin concentrations assayed in the samples, the CV of 9% for the five control samples was sufficient to allow adequate confidence for between-group comparison, which was the goal of the study. For agaritine, the results for the blinded triplicate analyses of one composite (UVB group, sample UV4) were 1.16, 1.03, and 1.01 mg/100 g, demonstrating a reasonable CV of 7.6%.

Proximate Composition. Results of proximate analysis of the mushroom composites, including moisture, ash, nitrogen, carbohydrate (by difference), and total fat, are presented in Table 2. Relative to the mushrooms processed in the presence or absence of UVB light, there was a slight (0.8%) but statistically significant ($p < 0.05$) reduction in the moisture content of the sun-exposed mushrooms, an effect that was likely due to dehydration occurring during the 2.5 h exposure period. The ash, nitrogen, and carbohydrate contents of the mushroom composites were consistent between groups on a dw basis, indicating that effective randomization of the crops and thorough homogenization of the samples was achieved.

Effect of UV Light and Sunlight on Vitamin D Content. As shown in Table 3, application of UVB light at an average exposure of 1.06 (0.03) J/cm^2 during processing produced mushrooms containing 410.9 (56.7) μg vitamin D_2/100 g on a dw basis. This represented an increase of 747% above the vitamin D_2 concentrations in the control mushrooms that were processed in the absence of UVB light. Sunlight-treated mushrooms contained vitamin D_2 at a level comparable to those processed with UVB light, with an average concentration of 374.5 (86.6) μg/100 g (dw). On the basis of respective moisture contents of 92.7 and 92.0% in the UVB- and sun-exposed mushrooms, an 84 g fresh weight serving (five medium-sized button mushrooms) of these mushrooms would provide 1008 (25 μg) and 918 IU (23 μg) of vitamin D_2, a nutritionally significant quantity that is sufficient to meet the current RDA of 600 IU (15 μg) for individuals aged 1−70 as recently established by the Food and Nutrition Board of the Institute of Medicine.[1] In contrast, the quantity of vitamin D in the same size serving of the control mushrooms was only 2% of the RDA for these individuals.

Unfortunately, the exact UVB doses attained during the 2.5 h sunlight exposure period could not be measured due to limitations in the sensitivity of the UV-monitoring device that were not appreciated before conduct of the experiment. With maximal UVB intensities of 250 μW/cm^2 at the equator, the lower sensitivity range (500 μW/cm^2) of the UV-monitoring device was clearly insufficient to measure UVB levels in central California during late summer. Exposure to long-wave UVA light could be measured, and over the 2.5 h exposure period an average dose of 24.0 (0.8) J/cm^2 of UVA light was measured. Although significant exposure to UVA light was recorded during the

Table 4. Water-Soluble Vitamin Content (per 100 g Dry Weight) of *Agaricus bisporus* Mushrooms Processed with UV Light[a]

		A. bisporus processed in the presence of light	
parameter	*A. bisporus* (control)	UVB	sunlight
vitamin C[b] (mg)	<14	<14	15 (3)
vitamin B_6 (mg)	0.76 (0.05)	0.71 (0.12)	0.72 (0.09)
riboflavin (mg)	3.37 (0.43)	3.51 (0.12)	2.48 (0.31)[c,d]
niacin (mg)	37.3 (3.5)	36.5 (1.0)	38.0 (2.5)
pantothenic acid (mg)	13.1 (1.8)	15.6 (2.1)	14.2 (1.8)
5-methyltetrahydrofolate (μg)	200.4 (72.9)	199.6 (62.8)	201.6 (51.4)
5-formyltetrahydrofolate (μg)	41.9 (6.6)	41.3 (13.6)	60.0 (5.9)[e]
10-formyl folic acid (μg)	20.4 (6.1)	16.2 (5.1)[f]	28.0 (8.1)

[a] Data are expressed as the mean (n = 5) followed by standard deviation in parentheses. [b] All samples except two in the sunlight group were below the limit of quantitation of 1 mg/100 g fresh weight (~14 mg/100 g dry weight). [c] p = 0.01 vs control. [d] $p < 0.001$ vs UVB. [e] $p < 0.05$ vs control; otherwise, means within each row did not differ significantly ($p > 0.05$). [f] The true average is expected to be lower because the analyte was detected in three samples at a concentration below the limit of quantitation of 1 μg/100 g fresh weight and a value of 1 μg/100 g fresh weight was used for estimating dry weight concentrations in these samples.

sunlight exposure treatment, the absorption spectrum for conversion of provitamin D (ergosterol) to vitamin D_2 ranges from 260 to 315 nm, which is outside the range of UVA light (315−400 nm); production of vitamin D from UVA light exposure would therefore be negligible.

A final note on the vitamin D_2 production achieved during incorporation of the UV lights into the commercial manufacturing process is the high level of reproducibility that can be attained using this technology. It has been reported that the orientation of the mushroom during processing can have a substantial effect on the quantity of vitamin D produced during exposure to UV light sources.[8,9] This effect has been attributed to regional differences in ergosterol content and morphological differences in the surface area subject to UV light exposure (gills vs cap). Regional differences in density of the mushroom flesh also may affect penetration of UV light into the tissue. During this experiment, the mushrooms were placed on the conveyor without regard to mushroom orientation and in a manner identical to that employed during a typical commercial production run. These exposure conditions produced mushrooms with highly reproducible vitamin D concentrations; the standard deviation of 56.7 μg/100 g (dw) indicates that an 84 g serving of mushrooms produced under these conditions would vary by 3.5 μg. This consistency exceeds that of many fortified products available on the marketplace. For example, a survey of vitamin D fortified milk samples obtained from the United States and Canada indicated that 80 and 73%, respectively, of the products sampled did not contain the minimum 80−120% range of vitamin D required for consistency with the product labeling. In some cases, products were absent vitamin D, and in other instances 2−3-fold excesses were reported.[7]

Ergosterol. Ergosterol is the principal sterol in mushrooms and is present in relatively high concentrations in *A. bisporus* (50−60 mg/100 g fresh weight).[2] The increase in vitamin D_2 concentration following exposure of mushrooms to UVB light or sunlight occurs via a well-described mechanism whereby ergosterol (provitamin D_2) is converted to previtamin D_2 via absorption of UV light energy within the 5,7-diene conjugated bond structure of the molecule. This absorption of UV light causes the isomerization of the molecule and bond cleavage between carbons 9 and 10, resulting in the unstable intermediate "previtamin D_2". The conversion of previtamin D_2 to vitamin D_2 then follows via a thermally catalyzed process.[7]

No significant difference in ergosterol concentration was detected between the UVB light treated and control mushrooms, which contained 579.5 (20.7) and 578.2 (29.8) mg/100 g (dw), respectively (Table 3). Although the production of vitamin D_2 was not associated with a significant decrease in ergosterol concentration, this finding is not unexpected given that the ergosterol concentrations exceed 500 mg/100 g of mushroom dw and the quantity of ergosterol converted to vitamin D_2 would be estimated to be a million-fold lower, a difference below that detectable by the analytical methodology.

An unexpected finding was the statistically significant increase in mean ergosterol concentration occurring in the sunlight-exposed group (633.4 mg/100 g dw), for which ergosterol concentrations were increased by 10% ($p < 0.01$) above both the control (578.2 mg/100 g dw) and UVB treatment groups (579.5 mg/100 g dw). The CV for n = 4 determinations of ergosterol in the mushroom control composites assayed with all samples was 0.84%. The explanation for the observed increase in ergosterol in sunlight-exposed mushrooms is unclear. The mushrooms used for this experiment were freshly harvested, and therefore would have been viable and biosynthetically active. Mushrooms are known for their high metabolic capacity and growth rate; induction of ergosterol synthesis following prolonged sunlight exposure may have occurred.

Effect of UVB and Sunlight on Vitamin C, Vitamin B_6, Riboflavin, Niacin, Pantothenic Acid, and Folate. For mushrooms processed in the presence of UVB light, other than the statistically significant increase in vitamin D, no substantial changes in vitamin concentrations were observed relative to the control groups (Table 4). In contrast to the findings in the UVB-exposed mushrooms, minor changes in riboflavin and 5-formyltetrahydrofolate concentrations were noted in the mushrooms exposed to sunlight. Relative to the control mushrooms processed in the absence of UV light, riboflavin concentrations were significantly reduced (−26%; $p < 0.01$) in the sunlight-exposed mushrooms. Because the degradation of riboflavin in the presence of sunlight is well established,[24] the observed modest reduction of riboflavin in the sunlight-exposed mushrooms is not unexpected.

A statistically significant increase in 5-formyltetrahydrofolate also was noted in the sun-exposed mushrooms (Table 4).

Table 5. Fatty Acid Content (Percent Dry Weight) of *Agaricus bisporus* Mushrooms Processed with UV Light[a]

		A. bisporus processed in the presence of light	
parameter	*A. bisporus* (control)	UVB	sunlight
fat by total fatty acid[b]	2.79 (0.11)	2.96 (0.13)	3.00 (0.14)
total saturated fatty acids	0.66 (0.06)	0.71 (0.07)	0.72 (0.09)
total monounsaturated fatty acids	0.14 (0.01)	0.14 (0.01)	0.15 (0.05)
total polyunsaturated fatty acids	1.86 (0.05)	1.97 (0.08)	2.07 (0.10)
4:0 butanoic acid	0.038 (0.015)	0.046 (0.006)	0.047 (0.010)
8:0 octanoic acid	0.005 (0.008)	0.006 (0.013)	0.000 (0.000)
10:0 decanoic acid	0.028 (0.011)	0.036 (0.008)	0.037 (0.014)
12:0 lauric acid	0.000 (0.000)	0.011 (0.018)	0.005 (0.011)
12:1 dodecenoic acid	0.019 (0.008)	0.016 (0.005)	0.032 (0.029)
14:0 myristic acid	0.017 (0.012)	0.016 (0.006)	0.010 (0.010)
14:1 myristoleic acid	0.014 (0.001)	0.011 (0.006)	0.010 (0.010)
15:0 pentadecanoic acid	0.011 (0.006)	0.019 (0.001)	0.025 (0.001)
16:0 palmitic acid	0.381 (0.033)	0.397 (0.042)	0.373 (0.018)
16:1 palmitoleic acid	0.003 (0.006)	0.000 (0.000)	0.008 (0.011)
17:0 heptadecanoic acid	0.008 (0.007)	0.011 (0.006)	0.013 (0.001)
18:0 stearic acid	0.096 (0.004)	0.104 (0.019)	0.117 (0.025)
18:1 oleic acid	0.066 (0.007)	0.063 (0.019)	0.062 (0.008)
18:2 linoleic acid	1.877 (0.041)	1.972 (0.069)	2.042 (0.103)
20:0 arachidic acid	0.036 (0.008)	0.038 (0.006)	0.040 (0.006)
22:0 behenic acid	0.019 (0.007)	0.022 (0.008)	0.025 (0.001)
24:0 lignoceric acid	0.022 (0.008)	0.027 (0.001)	0.025 (0.001)

[a] Data are expressed as the mean ($n = 5$) followed by standard deviation in parentheses. $p > 0.05$ for all between-group comparisons. [b] Sum of fatty acids as triglycerides.

Relative to the control mushrooms, 5-formyltetrahydrofolate concentrations were increased by an average of 43% (41.91 vs 60.0 μg/100 g respectively; $p < 0.05$). There is no evidence in the literature that transient sunlight exposure increases folate levels in mushrooms or other plants. However, all folates are to various degrees unstable and are particularly sensitive to oxidation.[25] Despite the fact that folates are good absorbers of UVB and UVA light, these compounds are believed to be relatively stable to UV light exposure, but degradation of 5-methyltetrahydrofolate in the presence of UV light has been reported in the presence of photosensitizers such as riboflavin.[26,27] In addition, 5,10-methylenetetrahydrofolate slowly rearranges to form 10-formyltetrahydrofolate, or 5-formyltetrahydrofolate, and as reported by Scott et al.,[25] 5-formyltetrahydrofolates are the most stable naturally occurring folate form. It is possible that the apparent increased concentration of 5-formyltetrahydrofolate may have occurred under oxidative conditions secondary to riboflavin photodegradation.

Why changes in riboflavin and 5-formyltetrahydrofolate were limited to sunlight exposure and not found in the UVB-exposed mushrooms is unclear, particularly given that the intensity of UVB light exposure would have been much higher than the UVB dose delivered via the sunlight exposure. However, UVA light is known to penetrate farther into tissues due to its longer wavelength, a property that may have increased the total impact of UV exposure on the sunlight-exposed mushrooms. In addition, there were large temperature differences between the UVB-processed and sunlight-exposed mushrooms, a consequence of the logistical requirement for the sunlight-exposed mushrooms to be processed outdoors. Mushrooms within the sunlight treatment groups were exposed to significantly higher ambient temperatures relative to the UVB and control mushrooms, which were processed in the mushroom packing facility that is maintained at a temperature of approximately 10 °C. During the 2.5 h sunlight exposure period, the internal temperature of the mushrooms reached 37.7 °C, an effect that may have catalyzed/enhanced any UV degradation reactions, potentially resulting in changes in riboflavin and 5-formyltetrahydrofolate.

Effect of UV Light on Fatty Acids and Amino Acids. The fatty acid content of the mushroom composites is presented in Table 5. The total fat content of the mushrooms was low (3 g/100 g dw on average in all treatment groups). The majority of fatty acids in the *A. bisporus* comprised linolenic (18:2) and palmitic acids (16:0), which constituted approximately 69 and 14% of the total fatty acids, respectively. Smaller amounts (<5%) of oleic (18:1−9*cis*) and stearic acid (18:0) were also present. Under the conditions of this experiment, exposure of *A. bisporus* mushrooms to UVB light or to sunlight did not affect the fatty acid content or composition of the mushrooms.

The mushrooms contained low quantities of amino acids (Table 6), with total protein low on a nutritional basis (<3 g/100 g fresh weight). Glutamic acid was the most abundant amino acid at approximately 3 g/100 g dw. Tryptophan, cysteine, and methionine were the least abundant, at levels below 0.5% (dw). Neither UVB nor sunlight altered the amino acid content or composition of the mushroom samples.

Effect on Agaritine. The concentrations of the phenylhydrazine alkaloid agaritine in the control mushrooms and in groups processed in the presence of UVB light or sunlight were 90.5 (43.8), 140.2 (56.2), and 110.5 (40.9) mg/100 g (dw), respectively. Significant within-group variability was noted for the

Table 6. Amino Acid Content (Percent Dry Weight) of *Agaricus bisporus* Mushrooms Processed with UV Light[a]

		A. bisporus processed in the presence of light	
amino acid	*A. bisporus* (control)	UVB	sunlight
tryptophan	0.41 (0.02)	0.41 (0.02)	0.38 (0.02)
cystine	0.14 (0.01)	0.14 (0.01)	0.13 (0.01)
methionine	0.41 (0.01)	0.41 (0.02)	0.38 (0.02)
aspartic acid	2.26 (0.53)	2.07 (0.41)	2.30 (0.35)
threonine	1.26 (0.12)	1.21 (0.12)	1.18 (0.12)
serine	1.23 (0.12)	1.21 (0.12)	1.18 (0.12)
glutamic acid	3.28 (0.53)	3.17 (0.58)	2.75 (0.28)
proline	1.31 (0.45)	1.52 (0.44)	1.35 (0.36)
glycine	1.23 (0.10)	1.27 (0.12)	1.18 (0.09)
alanine	2.55 (0.29)	2.76 (0.39)	2.38 (0.32)
valine	1.09 (0.17)	1.21 (0.19)	0.95 (0.06)
isoleucine	0.98 (0.12)	0.99 (0.08)	0.98 (0.10)
leucine	1.50 (0.18)	1.51 (0.18)	1.43 (0.16)
tyrosine	0.49 (0.08)	0.44 (0.08)	0.43 (0.08)
phenylalanine	0.99 (0.19)	0.85 (0.08)	0.88 (0.15)
total lysine	1.64 (0.22)	1.57 (0.11)	1.53 (0.12)
histidine	0.52 (0.06)	0.52 (0.08)	0.50 (0.08)
arginine	1.10 (0.33)	1.08 (0.43)	1.10 (0.09)

[a] Data are expressed as the mean ($n = 5$) followed by standard deviation in parentheses. $p > 0.05$ for all between-group comparisons.

analysis. On the basis of the quality control data for the blinded triplicate analyses of one sample, which yielded a CV = 7%, the within-group CV for each group (CV ~ 45%) suggests that agaritine levels in the *A. bisporus* may be naturally highly variable. Alternatively, agaritine is known to be very unstable, with pronounced degradation occurring in the presence of oxygen;[28] therefore, variability may have been introduced during thawing and secondary homogenization/processing of the composites. Nevertheless, the data are of sufficient quality to conclude that no large increases in agaritine were observed as a result of exposure to UVB or sunlight during processing. In addition, the agaritine concentrations measured in this experiment were within the range reported for other *A. bisporus* samples (94−629 mg/100 g dw), demonstrating that the agaritine levels in all groups are within the natural expected variation for agaritine in *A. bisporus*.[29,30]

As discussed, there are numerous reports on the use of UV light technology to produce vitamin D enhanced mushrooms. These studies have evaluated various UV exposure conditions using a variety of mushroom types and UV light sources. However, all of these studies have been conducted in benchtop laboratory settings that were not representative of commercial production processes and addressed only vitamin D and ergosterol. This is the first study to characterize the effect of UV light on the nutritional composition of mushrooms produced using commercial UV processing methods including vitamin D, ergosterol, and an analysis of the effect of exposure on components other than vitamin D and its precursors.

Conclusion. Overall, following an extensive analytical comparison of the effects of UVB and sunlight on *A. bisporus* white button mushrooms, it can be concluded that changes in mushroom composition as a result of exposure to UVB light under the commercial processing conditions described herein are limited to significant increases in the vitamin D content; no other nutritionally or toxicologically significant changes in mushroom composition were identified. The changes in vitamin D concentration occurring as a result of the commercial UVB light application were equivalent to those imparted by 2.5 h of sunlight exposure. Thus, the application of UVB light to mushrooms during commercial processing imparts material changes in the vitamin D content of mushrooms that are representative of those achievable within the natural environment.

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Notes

The study was funded by the U.S. Mushroom Council. R.R.S. and I.C.M. are employees of Cantox Health Sciences International, an Intertek Company (Cantox). Cantox has provided scientific consulting services to the U.S. Mushroom Council within the past three years.

ACKNOWLEDGMENT

We thank the staff at Monterey Mushroom Inc. for the generous donation of their time and for providing the mushroom samples used in the experiment, and in particular Kyon Kazemi, John Kidder, and Amada Lobato for their diligent work in processing the mushrooms. We acknowledge the work of Amy Rasor and Nancy Conley on the coordination and sample preparation of the mushroom composites, as well as the input of Mary Jo Feeney and Bart Minor in the study design and the writing of the paper.

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