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A Nutritionally Meaningful Increase in Vitamin D in Retail Mushrooms is Attainable by Exposure to Sunlight Prior to Consumption

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Abstract

The vitamin D_2 content of white button mushrooms is relatively low. UV exposure produces vitamin D_2 by rapid conversion of ergosterol to ergocalciferol. Commercial-scale UV treatment has been used to produce vitamin D-enhanced mushrooms. The reliability of a consumer-friendly protocol to increase vitamin D_2 in mushrooms by a nutritionally meaningful amount using exposure to sunlight was evaluated. Sliced white button mushrooms were exposed to sunlight for 15, 30, or 60 minutes in 16 experiments at different times of day, seasons, and cloud cover. Vitamin D_2 was measured by HPLC with 3H-vitamin D_3 internal standard. Change in vitamin D_2 per 70 g serving relative to untreated mushrooms was evaluated. Vitamin D_2 in all unexposed mushrooms was <30 IU/70 g (<5% DRI) (median, <7 IU/70 g). Regardless of season, treatment for 15 minutes between 9:30 a.m. and 3:30 p.m. under partly cloudy to clear conditions increased vitamin D_2 by157-754 IU/70 g (26-126%DRI), and up to 1142 IU/70 g total increase was observed after 30 min. On overcast and mostly cloudy days the gain was 76-178 IU/70 g (13-30%DRI) after 15 minutes, but after one hour the level was comparable to 15 minutes of treatment in clear conditions. Trials by consumers at four different geographic locations resulted in increases of 367-905 IU/70 g. A preliminary trial showed dramatically elevated vitamin D_2 contents in other mushroom types exposed 15 minutes under clear conditions. These results demonstrate that vitamin D_2 in mushrooms can be reliably enhanced by at least 25% of the DRI (150 IU; 3.75 µg)/70 g serving by exposure to sunlight for as little as 15 minutes on a clear or partly cloudy day between 9:30 a.m. and 3:30 p.m., and >100% (>600 IU) in many cases. Even under conditions of lower UV intensity similar increases can be achieved after 30-60 minutes.

Keywords: Vitamin D; Sun; Ultraviolet light; irradiation; Nutrient intake; Dietary supplements; Food composition; *Agaricus bisporus*; Shiitake; Oyster; Enoki

Introduction

While the role of vitamin D in bone health has long been recognized, recent attention has focused on its role in immune function and prevention of cancer and other diseases [1-13]. Vitamin D_3 (cholecalciferol) is produced naturally in humans from 7-dehydrocholesterol in skin exposed to ultraviolet light, but many individuals receive insufficient sun exposure to maintain adequate vitamin D status [5,14,15]. Living at latitudes >37°, vigilant use of sunscreen, dark skin color, minimal time spent outdoors, low consumption of vitamin D-rich foods and supplements, and age-related decline in the ability to synthesize the active form contribute to less than optimal vitamin D levels [5]. The serum concentration of 25-hydroxyvitamin D, a metabolite of vitamin D that increases with sun exposure and vitamin D intake, is generally accepted as an indicator of vitamin D status. It is estimated that 30-50% of individuals in Europe and the U.S. have insufficient levels [16-18]. Comprehensive reviews on the synthesis and metabolism of vitamin D are available [e.g., [5,19]].

The Food and Nutrition Board of the Institute of Medicine has established the Dietary Reference Intake (DRI) of 600 IU/day (15 µg) for children and adults up to age 70, and 800 IU (20 µg) for individuals >70 years of age [20,21]. The revised DRIs are a substantial increase relative to recommended adequate intake since 1997 of 200 IU (5 µg) per day for children and adults to up to age 50 y and 400 -600 IU (10-15 µg) for adults>50 years of age [22]. Other researchers recommend 1500-2000 IU/day as the adequate intake in individuals at risk for vitamin D deficiency [5,23]. The limited natural dietary sources of vitamin D_3 [(primarily fatty fish and fish oils [7]] and increasingly recognized suboptimal vitamin D status of individuals has created a need for alternatives to improve intake [24-26]. Dietary supplements and fortified foods are one means of delivery. In the U.S., fluid milk has been fortified with vitamin D since the 1930s [9], but more recently other foods have been approved for fortification, including breakfast cereal, orange juice, processed cheese, yogurt, margarine, and ice cream [27,28]. Most foods are fortified with vitamin D_3, but vitamin D_2 (ergocalciferol) is frequently used in dietary supplements and vegetarian products such as soy milk.

Vitamin D_2 is produced commercially by irradiation of yeast with ultraviolet light (UV), which converts the fungal sterol ergosterol to ergocalciferol in a process chemically analogous to the production of vitamin D_3 from 7-dehydrocholesterol [5,29,30]. The metabolism of vitamin D_2 to its biologically active form, 1,25-dihydroxyvitamin D_2, and the effectiveness of vitamin D_2 from supplements and edible mushrooms in improving vitamin D status has been reported [31-36]. Ergosterol occurs in milligram amounts in mushrooms, but microgram amounts of vitamin D_2 have biological activity (1 µg =40 IU), so conversion of a relatively small amount of ergosterol to vitamin D_2 results in a nutritionally significant increase in vitamin D.

All mushrooms contain ergosterol but the vitamin D_2 content varies widely. A recent study [37] found that in edible varieties of mushrooms

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in the U.S. retail market cultivated varieties [e.g., common white button, crimini, and portabella (*Agaricus bisporus*)] had nominal vitamin D_2 levels (<20 IU (0.5 µg) per 100 g) and wild grown types (e.g., morel, chanterelle) on average had higher contents [206-212 IU (5.15-5.30 µg) per 100 g]. The differences were not correlated with ergosterol content and were likely due to variable exposure to ambient UV. Mushroom producers have taken advantage of the conversion of ergosterol to vitamin D_2 by UV [29,33,38-41] to generate vitamin D enhanced mushrooms with commercial scale exposure to UV light. Vitamin D enhanced mushrooms are available in the U.S. [e.g., Sun Bella™ (Monterey Mushrooms, Watsonville, CA); Dole® Vitamin D Portobello mushrooms (Oakshire Mushroom Sales, LLC, Kennett Square, PA)]. The average vitamin D_2 content of enhanced portabella mushrooms sampled from U.S. retail markets was 448 IU (11.2 µg) per 100 g compared to only 10 IU (0.25 µg) per 100 g in untreated portabella [37].

Controlled studies have shown a large and rapid increase in vitamin D in mushrooms exposed to UV light [38,40,42]. For example, Roberts et al. [40] reported an increase of >1400 IU/100 g (>35 µg/100 g) in white button mushrooms after 8 minutes of UV-B irradiation at 0.5 J/cm^2, 1.0 mW/cm^2. Simon et al. [43] found vitamin D_2 increases in sliced white button mushrooms subjected to commercial scale UV treatment or 2.5 hr sunlight to be similar, going from 1.6 IU (0.4 µg) per 100 g in the untreated mushrooms to 1200 IU (30 µg) per 100 g for both methods of exposure. This content is 840 IU per 70 g serving and 140% of the DRI, compared to fortified milk with 100 IU (16.7% DRI) per 8 oz. (240 mL) serving [21]. Therefore it is reasonable to expect that exposure of mushrooms to sunlight for relatively short duration and/or submaximal UV intensity would result in a nutritionally meaningful increase in vitamin D content [e.g., ≥ 25% of the DRI (150 IU, 3.75 µg) per 70 g serving].

There are limited data on the consistency and magnitude of increases in vitamin D_2 that can be expected using ambient sun exposure, consisting of anecdotal reports [44,45]. A practical protocol for exposure of mushrooms to sunlight as a simple and inexpensive means to increase vitamin D intake was tested to evaluate the potential for generating a nutritionally significant increase in the vitamin D content of white button mushrooms using a consumer-based approach.

Methods

Study design

A series of experiments on sliced white button mushrooms (*Agaricus bisporus*) was conducted in Blacksburg, Virginia (U.S.A.) between March 2011 and March 2013 on different days and under varying conditions that affect UV exposure (e.g., cloud cover, season, time of day and duration of exposure), as summarized in Table 1. The approach was to use a consistent protocol that would be simple and practical for consumers to implement, and do repeated trials over a variety of conditions giving a wide range of UV exposure. A total of 16 experiments were done following the uniform protocol described below.

Samples and sample treatment

All sample preparation other than sun exposure was performed in

Expt.	Date	Season	Location*	Exposure conditions (post-exposure treatment)	UV index	Time of day	Surface		Exposure time (min.)		
									15	30	60
A	21-Mar-2011	Spring	Blacksburg, VA[b]	Partly cloudy	n.d.	1:37 pm	Aluminum pan				x
B	29-Jun-2011	Summer	Blacksburg, VA[b]	Clear	4 - 10	2:45 pm	Aluminum pan		x		
C	30-Aug-2011	Summer	Blacksburg, VA[b]	Clear	8 - 9	2:17 pm	Aluminum foil		x	x	
							White paper on glass pan		x		
							Dark non-stick pan		x		
							Aluminum pan		x	x	
D	12-Oct-2011	Fall	Blacksburg, VA[b]	Overcast	1 - 2	1:46 pm	Aluminum foil		x		x
E					6 -7	1:30 pm	Aluminum foil	Portabello, sliced	x		
					6 - 7	1:30 pm		Shiitake, sliced	x		
					6 - 7	1:30 pm		Enoki, whole	x		
					4 - 5	3:30 pm		White button, whole	x		
					4 - 5	3:30 pm		Oyster, sliced	x		
F	18-Oct-2011	Fall	Blacksburg, VA[c]	Partly cloudy	0 - 2	5:07 pm	Aluminum foil		x		
G	7-Mar-2012	Winter	Blacksburg, VA[b]	Clear	2 - 6	2:28 pm	Aluminum foil		x		
H	10-Mar-2012	Winter	Blacksburg, VA[b]	Clear	8 - 9	10:32 am	Aluminum foil		x		
I	6-Apr-2012	Spring	Blacksburg, VA[c]	Clear	2 - 7	4:07 pm	Aluminum foil		x		
J	7-Apr-2012	Spring	Christiansburg, VA[d]	Clear	7 - 9	11:15 am	Aluminum foil		x		
K1	4-Jun-2012	Summer	Dexter, MI[e]	Mostly clear	n.d.	11:00 am	Aluminum foil			x	
K2	9-Jun-2012	Summer	Corning, NY[f]	Mostly clear	n.d.	11:00 am	Aluminum foil			x	
K3	10-Jul-2012	Summer	Dallas, TX[g]	Mostly cloudy	n.d.	11:00 am	Aluminum foil			x	
K4	31-Jul-2012	Summer	Beltsville, MD[h]	Mostly clear	n.d.	10:45 am	Aluminum foil		x**		
L	6-Feb-2013	Winter	Blacksburg, VA[b]	Clear	4	12:52 pm	Aluminum foil		x		
M	25-Feb-2013	Winter	Blacksburg, VA[b]	Mostly cloudy	0 - 1	9:51 am	Aluminum foil		x	x	
N	14-Mar-2013	Spring	Blacksburg, VA[i]	Partly cloudy	2	9:09 am	Aluminum foil		x		

n.d.= not determined

*Latitude, longitude (elevation above sea level; orientation): [b]37.2264217, -80.4255651 (618m; east); [c] 37.2264217, -80.4255651 (618m; west); [d] 37.1438000, -80.4293000 (609m; east); [e] 42.329171, -83.872428 (277m; south-southeast); [f] 42.138641, -77.056551 (330m; north); [g] 33.03771, -96.836331 (210m, north-northwest); [h]39.032348, -76.911002 (42m; east); [i] 37.2432360, -80.4257903 (645m; southeast)

**18 min.

Table 1: Summary of parameters for experiments conducted. All experiments except E were with sliced white button mushrooms.

a laboratory with UV-filtered light. For each experiment, mushrooms (12-24 oz, usually 2-3 packages) were purchased locally within 24 hours of use. Sliced white button and sliced shiitake mushrooms were purchased pre-cleaned and used without further preparation. A soft nylon bristled brush was used to remove debris from whole white button and sliced portabella mushrooms, but no further trimming was done. Approximately 1.25 cm was trimmed from the bottom of whole enoki mushrooms and regarded as waste, and no further cleaning was done. Oyster mushrooms were purchased whole (pre-cleaned) and sliced immediately prior to the experiment. All mushrooms for a given experiment were combined and mixed thoroughly then subdivided into ~225 g quantities for a control (non-exposed) sample and ~225 g mushrooms to be subjected to each treatment. Mushrooms were spread on the surface (aluminum foil, aluminum pan, non-stick pan, or white paper) and placed outdoors in a non-shaded location. The UV index was measured with a UV meter (Oregon Scientific model EB612) in all experiments as an estimate of actual UV exposure. The control mushrooms for each experiment were homogenized in liquid nitrogen using a Robot Coupe® Blixer BX6V food processor (Robot Coupe USA, Inc., Jackson, MS) at the time the treated mushrooms began exposure. At the end of the treatment period the exposed the mushrooms were immediately homogenized in the same manner. All subsamples were dispensed into 2-oz wide-mouth straight-sided jars (Qorpak®, Bridgeville, PA), surrounded with foil, sealed with tape, and stored at -60°C in darkness until analyzed.

An experiment was done to compare results from exposure of sliced white button mushrooms placed on different surfaces [aluminum foil, an aluminum pan, a dark non-stick pan, and white paper (Table 1, experiment C)]. Based on the results of this experiment, aluminum foil (shiny side up) was used in the suggested protocol that was tested in the full series of experiments. The effect of storage on the vitamin D content of exposed mushrooms was also evaluated in one experiment (Table 1, experiment A) by placing a portion of treated mushrooms into an aluminum foil covered bowl in the refrigerator (2-5°C) overnight (24 hr) prior to homogenization and analysis.

Treatment of mushrooms by consumers at different geographic locations

After conducting experiments under different conditions of UV exposure in the same location in Blacksburg, trials were implemented by consumers at two homes within the same local area and at four homes in other geographic locations (Ann Arbor, MI; Corning, NY; Dallas, TX; Beltsville, MD) (Table 1, experiments K1-K4). Each individual was given a procedure to follow which replicated the method used in the experiments in Blacksburg. Sliced white button mushrooms (12-16 oz.) were purchased locally by the participants during June-July 2012. After setting aside half of the mushrooms as a non-exposed control, each participant exposed the mushrooms between 10:30 and 11:30 a.m. local time for 18-30 min. on aluminum foil. The control and treated mushrooms were frozen overnight in separate Rubbermaid® containers in home freezers, then shipped to Blacksburg the next day on ice packs via priority express overnight delivery. Composites were prepared and analyzed for moisture and vitamin D_2 as described for the on-site experiments.

Evaluation of different mushroom types

In a separate experiment (E, Table 1), whole white button, sliced portabella, sliced shiitake (*Lentinus edodes*), sliced oyster (*Pleurotus ostreatus*), and whole enoki (*Flammulina velutipes*) mushrooms were exposed at the same time and following the same protocol as for sliced white button mushrooms, to provide preliminary data on increases in vitamin D_2 that could be expected in different types of mushrooms. Mushrooms were locally purchased and treated using the procedure described for sliced white button mushrooms, with exposure on aluminum foil for 15 minutes on a clear day (17-Oct-2011, UV index 4-5) at 1:30 p.m. in Blacksburg, VA at the same location as for the repeated experiments with sliced white button mushrooms. Composites were prepared and analyzed for vitamin D_2 as described for sliced white button mushrooms.

Analysis of vitamin D_2

Vitamin D_2 was extracted and analyzed by high performance liquid chromatography (HPLC) at Heartland Assays, Inc. (Ames, IA) using methodology previously reported [37]. Briefly, mushroom samples with [^{3}H]-vitamin D_3 added as an internal standard were saponified in methanolic KOH, purified by solid-phase extraction and HPLC to isolate the vitamin D fraction, and vitamin D_2 was then separated by reverse-phase HPLC with UV detection at 265 nm and quantified based on the ratio of sample peak area to [^{3}H]-vitamin D_3 ratio relative to an external standard curve from analysis of vitamin D_2 standards spiked with an equivalent amount of internal standard.

Quality control and statistical analyses

Each composite was assayed in blinded duplicate or triplicate, and a blinded sample of a mushroom control composite ("Mushroom CC", a mixture of portabella and vitamin D enhanced portabella mushrooms) was included in each analytical batch, with the analyst blinded to the identity of replicate samples. The Mushroom CC had been previously analyzed and had an established confidence interval for vitamin D_2 [37] and was the same control used in previous reports [37,43]. The value for the Mushroom CC in each batch was compared to established tolerance limits, and if it fell outside the range all samples in the batch were rerun. Moisture in each composite was determined by vacuum drying to a constant weight at 60°C and 25 mm Hg [46].

The outcome variable was increase in vitamin D_2 per 70 g (1 cup) serving, calculated as the assayed vitamin D_2 content of treated mushrooms minus the mean vitamin D_2 content of corresponding untreated mushrooms), when there was a statistically significant difference in the assayed mean for the treated and untreated mushrooms. Means and standard deviations were calculated using Microsoft® Office Excel (Professional edition 2010; Microsoft Corporation, Redmond, WA). Analysis of variance (α=0.05) and pairwise comparison of means using the Tukey test [47] with a 95% confidence interval were performed using XLSTAT (version 2012.4.03; Addinsoft, New York, NY). The relationship between UV index and increase in vitamin D_2 was evaluated through bivariate regression analysis of exposure time x UV index as the independent variable, using the JMP 10.0.2 software package (SAS Institute Inc., Cary, NC).

Results

Quality control

The vitamin D_2 concentration in the Mushroom CC included in each batch of samples assayed (n=10) ranged from 244-296 IU/100 g [mean, 273; median, 272; relative standard deviation (RSD), 6.2%]. All values fell within the mean ± 2*standard deviation of previous results obtained (Figure 1). The Mushroom CC samples were stored in the same type of containers and under the same conditions as the mushroom samples in this study prior to analysis, and the consistency of the assayed vitamin D_2 concentration over a 3.8 year period demonstrates stability of the

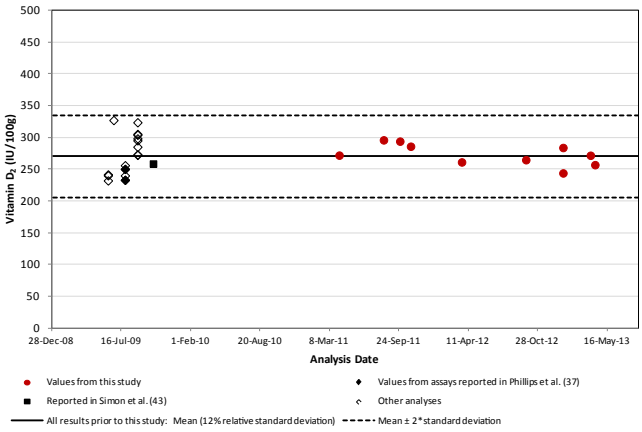

Figure 1: Results for samples of Mushroom Control Composite analyzed in this study compared to previous results, over a 3.8 year period.

Experiment (Table 1)	Vit D_2 (IU/70 g serving)[a]		
A	27.7	±	2.5
B	11.2[b]		
C	10.5	±	4.9
D	3.4	±	4.1
F	3.8	±	0.5
G	< 7		
H	< 7		
I	< 7		
J	< 7		
K1	< 4		
K2	< 4		
K3	< 4		
K4	11.7	±	2.9
L	23.0	±	21.0
M	12.8	±	8.0
N	8.0	±	1.3

[a]Mean ± standard deviation
[b]not applicable (n=1)

Table 2: Vitamin D_2 content of untreated sliced white button mushrooms in experiments described in Table 1.

nutrient in the samples. The analytical precision achieved is sufficient to allow confidence that data from separate analytical batches, that also contained samples from different experiments, did not differ due to significant analytical variability. Further, the results for the Mushroom CC were consistent with those from analyses done as part of other published studies on vitamin D in mushrooms [37,43], allowing confidence in comparing results for samples among these studies.

Sliced white button mushrooms exposed under varying conditions

The vitamin D_2 content of unexposed sliced white button mushrooms in all experiments (n=16) was <30 IU/70 g (< 5% DRI), with a range of <3 to 28 IU/70 g and a median of <7 IU/70 g (< 1.2% DRI) (see Table 2). The difference in the mean assayed vitamin D_2 concentration in the untreated and treated mushrooms was statistically significant ($p<0.05$) in all experiments and exposure times shown in Table 1.

Figure 2 illustrates these increases in vitamin D_2 for sliced white button mushrooms. Results of means comparisons are given in Table 3. For experiments in the Blacksburg area under varying conditions of UV exposure (experiments A-J and L-N, Table 1), increases of <25% of the DRI occurred only in experiments N, F, and M (7.3, 24, and 78 IU/70 g, respectively) after 15 minutes exposure on partly to mostly cloudy days before 10:00 a.m. (N and M) or after 5 p.m. (F), as indicated in Table 1. With 15 min. additional exposure, however, the mushrooms with a level of 78 IU/70 g in experiment M (mostly cloudy day, winter, 9:51 a.m., UV index 0-1) had a total increase of 168 IU/70 g (28% DRI).

Considering all other treatments that resulted in >150 IU/70 g increase in vitamin D_2, for samples exposed during the mid-day (9:30 a.m.-3:30 p.m.) on clear days, regardless of season (experiments B, C, G, H, I, L), vitamin D_2 rose 157-754 IU/70 g (26-126%DRI) after 15 minutes, and (in experiment C) another 271-438 IU/70 g after an additional 15 minutes. On an overcast day (fall season, mid-day; experiment D) vitamin D_2 increased by 178 IU/70 g (30%DRI) after 15 minutes exposure, and by 510 IU after 1 hour, which was within the range in mushrooms exposed 15 min. on clear days.

Table 4 summarizes the change in vitamin D_2 in sliced white button mushrooms placed on different surfaces (aluminum pan, aluminum foil (shiny side up), non-stick pan, or white paper) and exposed to sunlight mid-day with clear skies (Table 1, experiment C). After 15 minutes, vitamin D_2 increased from only 10.5 IU/70 g in the unexposed mushrooms to 661-765 IU/70 g. The change of 651 IU/100 g in mushrooms exposed on white paper was slightly lower than in the mushrooms treated on the other surfaces ($p=0.038$), with no measured difference between the others. The change was dramatic in all cases, taking the mushrooms from providing < 2% of the RDA to >110% per serving. An additional vitamin D_2 increase of 271-439 IU/70 g resulted after a total of 30 minutes exposure for the aluminum pan and aluminum foil surfaces, yielding 1035-1153 IU/70 g (172-192%DRI).

In the comparison of the vitamin D_2 content of treated mushrooms immediately after exposure and after storage overnight in the refrigerator (24 hours), the concentration did not change significantly from the level of 738 IU/70 g immediately after exposure ($p>0.05$). This is not unexpected, given the <10% loss of vitamin D_2 in cooked mushrooms

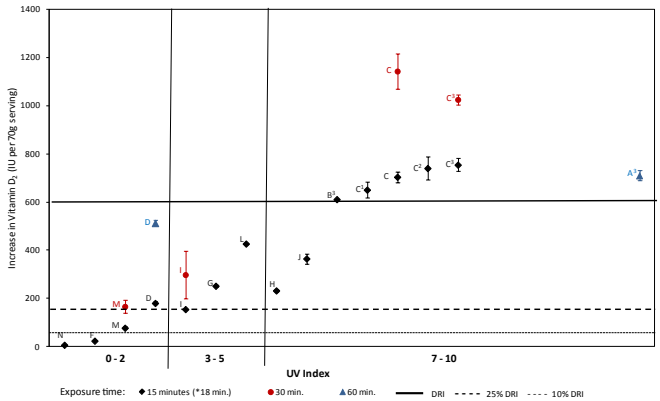

Figure 2: Increase in vitamin D_2 in sliced white button mushrooms exposed to sunlight under varying conditions of UV intensity (mean ± standard deviation; 1 IU = 0.025 µg). Vitamin D_2 in the control (unexposed) mushrooms was < 30 IU/70 g (Table 3) and differed significantly from the corresponding exposed mushrooms in all cases ($\alpha=0.05$). Letters on data points correspond to experiments in Table 1. Data points with no superscript were for mushrooms placed on aluminum foil, and superscripts indicate mushrooms exposed on [1]white paper, [2]dark non-stick pan, and [3]aluminum pan. DRI= Dietary Reference Intake (20). See Table 3 for results of means comparisons.

Experiment[a]	LS means	Groups											
C (30 min)	1142	A											
C* (30 min)	1025	A	B										
K2	903		B										
C* (15 min)	754			C									
C***	740			C									
A* (60 min)	710			C									
C (15 min)	704			C									
K4	703			C									
K1	695			C									
C**	651			C									
B*	611			C	D								
D (60 min)	512				D	E							
L	427					E	F						
K3	387					E	F	G					
J	365						F	G	H				
I (30 min)	298						F	G	H	I			
G	252							G	H	I			
H	233								H	I			
D (15 min)	181									I	J		
M (30 min)	167									I	J	K	
I (15 min)	155									I	J	K	
M (15 min)	78										J	K	L
F	24.3											K	L
N	7.3												L

Table 3: Results of least square (LS) means comparison of increases in vitamin D_2 (IU/70g) in mushrooms exposed under different conditions (Table 1 and Figure 2). Increases in vitamin D for experiments denoted by same capital letter for Group do not differ significantly (α=0.05). All treatments were done on an aluminum foil surface except those marked with * (aluminum pan), ** (white paper on glass pan), and *** (dark non-stick pan).

		Increase in Vitamin D_2 (per 70 g serving)			
Surface	Exposure time (min.)	IU[a]			Percent of DRI[b]
Aluminum pan	15	754	±	27[A]	126
	30	1025	±	21[B]	171
Aluminum foil	15	704	±	22[A]	117
	30	1142	±	73[B]	190
Dark non-stick pan	15	740	±	49[A]	123
White paper on glass pan	15	651	±	33[A]	108

[a]Mean ± standard deviation of 2 or 3 replicate analyses. Means that differ significantly (α=0.05) are indicated by different superscripts. 1 IU=5 µg
[b]Dietary Reference Intake, 600 IU/day [20].

Table 4: Increase in vitamin D_2 in sliced white button mushrooms placed on different surfaces and exposed to sunlight on a clear day (Experiment C, Table 1). Vitamin D_2 in the control (unexposed) mushrooms was 10.5 ± 4.9 IU per 70 g serving, and all increases were statistically significant (p<0.0001).

and no decrease after 9 months storage at -20°C demonstrated by Mattila et al. [48] for chanterelle mushrooms.

Prediction of increase in vitamin D_2

UV light causes the conversion of ergosterol to vitamin D_2, and increases in vitamin D_2 in mushrooms exposed to UV light are related to the UV dose [40]. Parameters that affect UV intensity (including latitude, ozone and smog, elevation) and that would vary in generalized implementation of consumer-initiated treatment of mushrooms were not possible to capture in this study due to practical constraints. However, a wide range of ultimate UV dose received by treated mushrooms was achieved using variation in parameters that were possible to control (Table 1). The regression equation for increase in vitamin D_2 IU per 70 g serving (ΔD_2) as a function of UV index and exposure time (minutes), assuming no interaction between UV index and exposure time and using data from experiments conducted in the Blacksburg area locations using aluminum foil (experiments C-J and L-N), was:

$$\Delta D_2 = 3.10 + 3.92 * (\text{UV index} * \text{exposure time})$$

The R-square coefficient was 0.74, suggesting that UV index and

time explain 74% of the change in vitamin D_2. In turn, this suggests a reasonable degree of confidence in using these parameters to predict the increase in vitamin D_2 in any particular trial. Theoretically, the variation in UV index reported in the literature, or measurement of the UV index at a particular location where mushrooms are exposed could be used to predict outcomes at wide-ranging locations, times of day, seasons, and ambient conditions. While the UV index is not an absolute measure, the data and specification of equipment used to measure "UV index" in this study provide a basis for generalizing results.

It should be noted that practical exposure times, from a consumer perspective, were chosen based on the goal of this work. Also, exposure in full sun for longer than 30 minutes could be expected to affect the quality of the fresh mushrooms. For 60 min. exposure times, some withering and darkening of mushrooms was observed (experiment B).

Mushrooms treated by consumers at different geographic locations

In the trials completed by consumers at different locations (Table 1, experiments K1, K2, K3, K4) in which mushrooms were placed in the sun starting between 10:45 and 11:00 a.m. local time, vitamin D_2 increased by 367-905 IU per 70 g serving (61-151%DRI) after 30 min. (K1, K2, K3) or 18 min. (experiment K4). The UV index was not measured by consumers, but assuming UV indices of 7 and 3 for the reported "mostly clear" and "mostly cloudy" conditions, the predicted increase in vitamin D_2 based on the regression analysis of results from local experiments was calculated. As illustrated in Figure 3, in all cases but experiment K4, the increase was within 10% of the predicted amount, while in experiment K4 the increase exceeded what was predicted. Although these analyses are preliminary, they suggest it might be possible to predict the increase in vitamin D_2 in a particular location by estimating the UV index at that location relative to Blacksburg, VA. A number of articles on monitoring and prediction of UV index and relative UV exposure at different latitudes and relative to other factors have been published [49-59]. Similarly, models for estimating cutaneous UV exposure at different geographic locations as it relates to production of vitamin D_3 [60] might prove useful in generalizing the results in this study for vitamin D_2 production in mushrooms exposed to ambient UV at different locations.

Other mushroom types

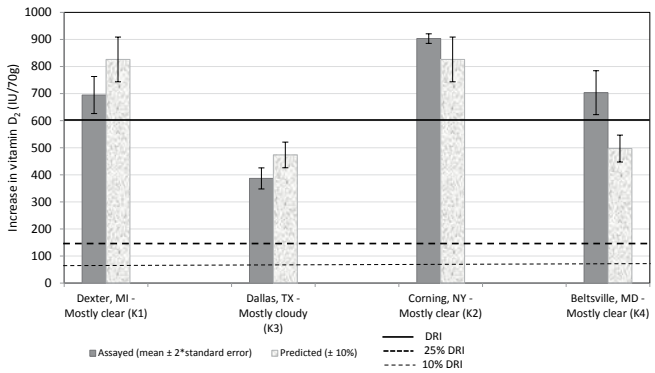

Figure 3: Increase in vitamin D_2 content of sliced white button mushrooms exposed to sunlight by consumers at different geographic locations compared to predicted increase
(3.10 + 3.92*UVindex*exposure time), assuming UV indices of 7 and 3 for "mostly clear" and "mostly cloudy" conditions, respectively. Letters in parentheses in legend refer to corresponding experiments in Table 1. DRI= Dietary Reference Intake [20].

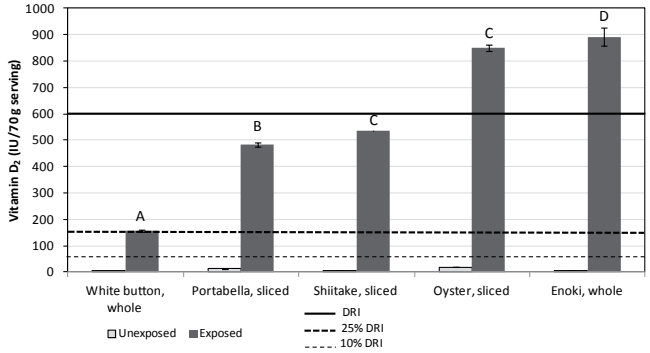

Figure 4: Change in vitamin D_2 content of different types of mushrooms exposed to sunlight for 15 minutes on a clear day (mean ± standard deviation). Different capital letters indicate means that different significantly (α=0.05). DRI=Dietary Reference Intake [20].

Figure 4 shows the change in vitamin D_2 content of whole white button, sliced shiitake, sliced oyster, and whole enoki mushrooms exposed to sunlight for 15 minutes mid-day, with clear skies (Table 1, experiment E). Whereas the vitamin D_2 content of all of the unexposed mushrooms was low (< 7 to 18.2 IU/70 g), the level increased after treatment in all types. The largest gains were in oyster and enoki (832 and 886 IU/70 g, respectively). Lower but still dramatic increases resulted in portabella and shiitake (472 and 529 IU/70 g, respectively). In whole white button mushrooms the increase was 154 IU/70 g, which was lower than in the other mushroom types and the range of 424-704 IU/70 g in sliced white button mushrooms exposed at the same location under similar conditions (experiments B, C, H, L; Table 1 and Figure 2). The lower increase is consistent with studies by Jasinghe and Perera [61] that showed using experiments with controlled UV exposure that orienting mushrooms with the gills facing the UV source resulted in higher vitamin D levels, given the higher surface area of gills exposed in the sliced mushrooms. Interestingly, however, in whole enoki mushrooms the vitamin D increase was substantially higher than in all other types of sliced mushrooms except oyster. It is important to note, however, that even in the whole white button mushrooms just 15 minutes sun exposure resulted in a 14.7% increase in the DRI contributed by a 70 g serving, which is three times the vitamin D provided by 8 oz. of fortified milk.

Conclusion

Exposure of sliced white button mushrooms to sunlight proved to be a simple, reliable, and convenient method by which consumers could increase vitamin D intake. Treatment for as little as 15 minutes on a clear or partly cloudy day consistently increased vitamin D_2 by at least 25% of the DRI (150 IU) per 70 g serving, and more than 100% of the DRI (> 600 IU) in many cases. Figure 5 shows the range in vitamin D_2 content of sliced white button mushrooms exposed to sunlight for 15 minutes in this study compared to the levels in retail samples of different types of mushrooms and to the vitamin D_3 content of other foods. Even with low UV exposure (UV index ≤ 2), arising from factors such as non-optimal orientation to sun, mostly cloudy and overcast conditions, etc., the vitamin D content increased to be comparable to the level in other fortified foods, and was substantially greater after longer exposure times. Preliminary results also suggested the same effects are possible with other types of mushrooms, with possibly greater effects of UV in oyster and enoki. Even though for practical reasons the protocol could be tested at limited geographic locations,

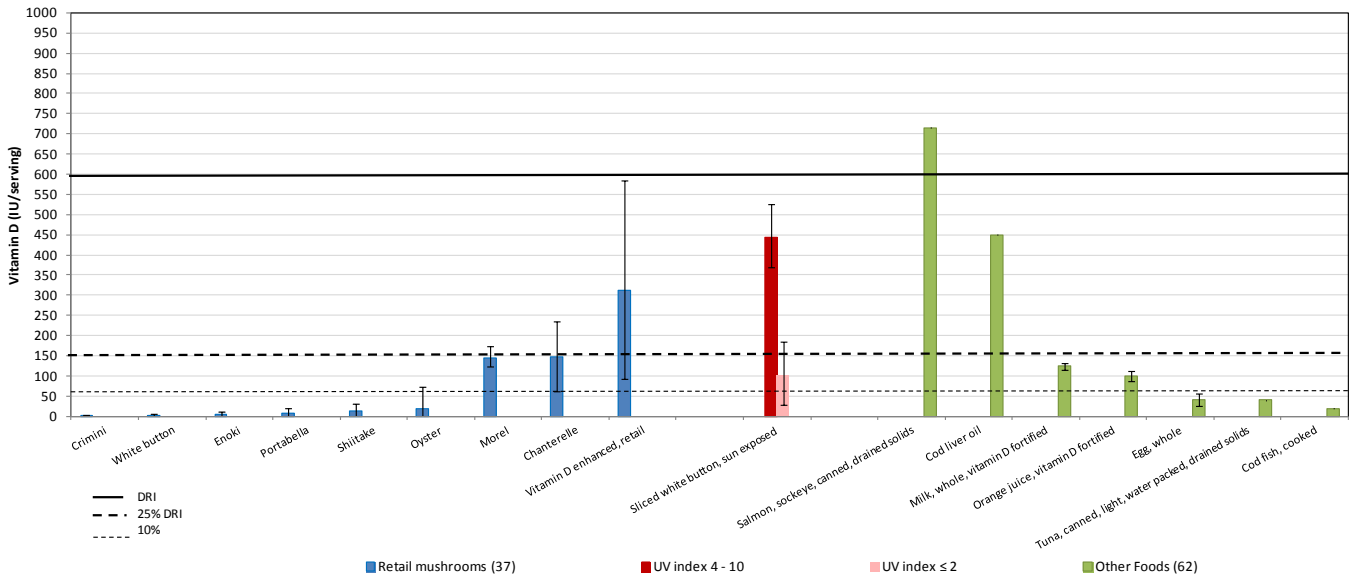

Figure 5: Vitamin D_2 content of sliced white button mushrooms exposed to sunlight between 9:30 a.m. and 3:30 p.m. Eastern Standard Time, compared to vitamin D_2 in retail samples of different mushroom types [37] (mean ± range), and to vitamin D_3 in other foods (mean ± standard error) [62]. DRI=Dietary Reference Intake [20].

UV index and exposure time explained 74% of the changes in vitamin D_2 in the experiments conducted and provides a basis to estimate effects at other geographic locations and conditions (e.g., higher or lower latitudes, higher elevations, different ozone levels) based on UV index. An investment of as little as 15 minutes could provide a low-cost and convenient consumer-based strategy to increase dietary vitamin D intake.

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