JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Vitamin D and Sterol Composition of 10 Types of Mushrooms from Retail Suppliers in the United States

Katherine M. Phillips,[*,†] David M. Ruggio,[†] Ronald L. Horst,[§] Bart Minor,[#] Ryan R. Simon,[⊥] Mary Jo Feeney,[⊗] William C. Byrdwell,[△] and David B. Haytowitz[□]

[†]Virginia Tech, Blacksburg, Virginia 24061, United States

[§]Heartland Assays, Inc., Ames, Iowa 50010, United States

[#]Mushroom Council, San Jose, California 95134, United States

[⊥]Cantox Health Sciences International, Mississauga, Ontario, Canada L5N 2X7

[⊗]Consultant to Food and Agricultural Industries, 11030 Mora Drive, Los Altos, California 94024, United States

[△]Food Composition and Methods Development Laboratory and [□]Nutrient Data Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, United States

ABSTRACT: Vitamin D_2 (ergocalciferol) and sterols were analyzed in mushrooms sampled nationwide in the United States to update the USDA Nutrient Database for Standard Reference. Vitamin D_2 was assayed using HPLC with [^{3}H]-vitamin D_3 internal standard and sterols by GC-FID mass spectrometric (MS) confirmation. Vitamin D_2 was low (0.1−0.3 μg/100 g) in *Agaricus bisporus* (white button, crimini, portabella) and enoki, moderate in shiitake and oyster (0.4−0.7 μg/100 g), and high in morel, chanterelle, maitake (5.2−28.1 μg/100 g) and UV-treated portabella (3.4−20.9 μg/100 g), with significant variability among composites for some types. Ergosterol (mg/100 g) was highest in maitake and shiitake (79.2, 84.9) and lowest in morel and enoki (26.3, 35.5); the range was <10 mg/100 g among white button composites but 12−50 mg/100 g among samples of other types. All mushrooms contained ergosta-5,7-dienol (22,23-dihydroergosterol) (3.53−18.0 mg/100 g) and (except morel) ergosta-7-enol. Only morel contained brassicasterol (28.6 mg/100 g) and campesterol (1.23−4.54 mg/100 g) and no ergosta-7,22-dienol. MS was critical in distinguishing campesterol from ergosta-7,22-dienol.

KEYWORDS: chanterelle, crimini, enoki, maitake, morel, oyster, shiitake, white button, *Agaricus bisporus*, *Morchella*, *Cantharellus*, *Lentinus edodes*, *Flammulina veluptipes*, *Grifola frondosa*, *Pleurotus ostreatus*, phytosterols, brassicasterol, campesterol, ergosterol, vitamin D_2, ergocalciferol, ergost-7-enol, ergosta-5,7-dienol, ergosta-7,22-dienol, 22,23-dihydroergosterol, fungisterol, mass spectrometry, GC-FID, GC-MS, HPLC

INTRODUCTION

Vitamin D has been well established as critical for bone health. More recently its role in immune function and prevention of some types of cancer and other diseases has received increasing attention.[1−4] Vitamin D_3 (cholecalciferol) is produced from 7-dehydrocholesterol in the skin during exposure to ultraviolet (UV) radiation; however, individuals with inadequate sun exposure rely on vitamin D from foods and supplements to obtain an adequate supply of this essential nutrient. New updated dietary reference intakes (DRI) for vitamin D were recently published by the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM)[5] with a recommended dietary allowance (RDA) of 600 IU (15 μg) for children and adults up to age 50 and 800 IU (20 μg) for individuals >70 years of age. These RDA represent an increase relative to the previous recommended adequate intakes (AI) from 1997 of 200 IU (5 μg) per day for children and adults up to age 50 and 400−600 IU (10−15 μg) for adults ages 50−70 and >70.[6,7] Vitamin D in foods occurs naturally primarily as D_3 and 25-hydroxy-vitamin D_3 in animal products. Milk, fortified cereals, and an increasing number of other foods, including juices, processed cheese, yogurt, margarine, and ice cream, may be fortified with vitamin D, usually as D_3 in the United States, typically at 2.5 μg (100 IU) per 8 oz (240 mL) serving of milk. Nonanimal foods contain vitamin D_2 (ergocalciferol), mostly at lower levels. Some types of mushrooms are rich natural food sources of vitamin D_2; concentrations of 0.3−59 μg/100 g fresh weight have been reported in some common edible varieties.[8] Therefore, the contribution of mushrooms to total vitamin D intake could be significant, depending on the amount and type consumed. Many different species of mushrooms are consumed worldwide, and they represent a rich natural food source of this vitamin for individuals having a diet devoid of vitamin D-fortified foods, not taking dietary supplements, and/or with inadequate sun exposure.

Vitamin D_2 in mushrooms is produced upon exposure to sunlight and other sources of UV light through the photosynthetic conversion of ergosterol to vitamin D_2 via production of unstable previtamin D_2 intermediates (tachysterol, lumisterol), similar to the photochemical reaction in human skin, where 7-dehydrocholesterol is converted to vitamin D_3 after passing through the unstable intermediate previtamin D_3.[9] When

Received: November 3, 2010
Revised: June 10, 2011
Accepted: June 13, 2011
Published: June 13, 2011

consumed, vitamin D_2 is metabolized to a biologically active form, 1,25-dihydroxy-vitamin D_2, via the intermediate 25-hydroxy-vitamin D_2. The bioavailability of vitamin D_2 from mushrooms has been reported,[10,11] and comprehensive reviews have been published on the synthesis and metabolism of vitamin D in humans, plants, and fungi.[9,12−15]

Intentional UV treatment of ergosterol in vitro and in yeast, a rich source of ergosterol, has a long history of commercial use for the production of vitamin D_2 for dietary supplements, pharmaceutical grade vitamin D preparations, and food fortification.[16] Recently, this same technology has been employed by mushroom producers, who have taken advantage of the fact that, like yeast, mushrooms are a rich source of ergosterol, and UV light can be used to produce vitamin D enhanced mushrooms; these mushrooms are now available in some U.S. retail markets. All species of commercial mushrooms have the capacity to produce nutritionally significant quantities of vitamin D upon exposure to UV light, and depending on the experimental conditions, the process can occur rapidly, requiring in some cases only seconds of light exposure. The effects of UV light on vitamin D production among a wide variety of mushroom species and exposure conditions have been recently reported by a number of authors.[8,11,17−20]

Consumption of phytosterols has been shown to be hypocholesterolemic,[21] and phytosterols compete with cholesterol for absorption from the intestine.[22] Consumption of mushrooms has specifically been studied in animal models and cell systems for hypocholesteromic and antiatherosclerotic effects[23−25] and also anticarcinogenic properties.[26] Ergosterol was a primary component in extracts exhibiting these effects in some studies,[24,26] although it has not been proven or disproven to be the biologically active component. To carry out epidemiological studies and controlled feeding trials evaluating relationships of dietary intake of noncholesterol sterols to health and disease, food composition data for noncholesterol sterols are required. Adequate food composition data are required before dietary recommendations can be formed and to identify potential sources of phytosterols for commercial applications.

Researchers have reported the vitamin D_2 and/or sterol content of a number of mushroom species (see, for example, Teichmann et al.,[8] Jasinghe and Perera,[17] Senatore,[27] Yokokawa and Mitsuhahi,[28] Mallavadhani et al.,[29] Mattila et al.,[30−32] and Mallaadhani et al. [33]). However, data are lacking on sample-to-sample variability among species and on the content and variability of vitamin D and sterols in mushrooms in the United States. Of interest is the potentially wide variability in vitamin D content, both within species and among samples of the same species, resulting from differences in naturally occurring ambient light exposure during harvest, processing, and handling prior to consumption. In commercially produced UV-treated mushrooms, orientation of the mushroom gills toward the light source, the wavelength, intensity, exposure time, moisture content, and mushroom species affect the concentration of vitamin D_2 that can be produced.[18,19] Although the vitamin D_2 produced following UV irradiation has been reported to gradually diminish over time, further research is required because recent studies have presented conflicting findings.[11,20] Other factors during growth, storage, and distribution might also affect the vitamin D and sterol content of wild-grown and cultivated varieties of mushrooms.

To provide vitamin D and sterol composition data for the U.S. Department of Agriculture (USDA) National Nutrient Database for Standard Reference (SR),[34] 10 types of mushrooms, including commercially produced UV-treated portabella, were sampled as part of the National Food and Nutrient Analysis Program (NFNAP)[35] in cooperation with the Mushroom Council (San Jose, CA) and analyzed for vitamin D_2, ergosterol and ergosterol metabolites, and phytosterols. SR is the primary source of U.S. food composition data for estimation of nutrient intake from food consumption data. The vitamin D data from this study were incorporated in release 22 of SR,[34] included in version 4.1 of the Food and Nutrient Data set for Dietary Surveys used in the What We Eat in America component of the National Health and Nutrition Examination Survey,[36] facilitating research requiring increased knowledge of the vitamin D content of the U.S. diet. The phytosterol values were included in release 23 of SR in 2010.

MATERIALS AND METHODS

Samples. Mushrooms were procured in April 2009 according to a statistical plan designed to complement an earlier sampling in November 2004 by including two additional types not previously collected (morel and chanterelle) as well as commercially distributed portabella mushrooms produced with exposure to UV light to increase the vitamin D content. The 10 mushroom types sampled included chanterelle, crimini, enoki, maitake, morel, oyster, portabella, vitamin D-enhanced portabella (UV-treated), shiitake, and white button. They were sampled from retail markets in 12 U.S. cities according to a statistically representative plan developed for the NFNAP[37,38] and procured as described elsewhere.[39] For a few types (shiitake, oyster, and crimini) that could not be found in all retail locations, samples were either procured directly from producers or purchased at local retail markets (Blacksburg, VA). Samples (0.6−3 kg each) of enoki, maitake, chanterelle, morel, and vitamin D-enhanced portabella mushrooms were obtained from at least two producers. For the samples procured from retail outlets, 200−1020 g per mushroom type (either loose or packaged) was purchased from each market. The mushroom samples were packaged in perforated coolers containing freezer packs, taking care to avoid contact between the samples and the freezer packs, and shipped via overnight service to the Food Analysis Laboratory and Control Center at Virginia Tech (Blacksburg, VA). Upon receipt, the product label and visual appearance were used to verify sample identification. All samples were refrigerated (2−8 °C) between receipt and compositing/homogenization, with storage time ranging from <1 to 6 days.

For each mushroom type, the 12 samples from all locations were randomly assigned to one of four composites, with approximately 200−500 g from each retail outlet, with equal weight (±50 g) from each outlet and no subsampling of individual mushrooms. For samples shipped directly from suppliers, approximately 0.5−2.3 kg was used for each composite, depending on the amount of product provided. Samples from different producers were not combined, although separate lots from each producer were composited together.

Preparation and homogenization of each composite were performed in a UV-protected environment. At the time of compositing, mushrooms were removed from their packaging, placed on a lint-free cloth, and gently brushed with a kitchen brush and wiped with a lint-free cloth dampened with distilled, deionized water to remove excess debris. The mushrooms were then placed on a cutting board and trimmed of inedible/damaged portions and the stem base using a stainless steel knife. For portabella mushrooms only, the entire stem was removed and discarded. After cleaning and trimming, the mushrooms were cut into pieces of ∼1.25 cm, frozen in liquid nitrogen, and then homogenized using a 6 L stainless steel industrial food processor (Robot Coupe Blixer, Robot Coupe USA, Jackson, MS) while being kept frozen with liquid nitrogen. Subsamples (10−15 g each) of the frozen composite were dispensed into 60 mL glass jars with Teflon-lined lids, surrounded with aluminum foil, and stored in darkness at −60 °C prior to analysis (within 10−16 weeks).

A mushroom control composite (mushroom CC) for use as an analytical quality control material was also prepared. The mushroom CC comprised a total of 2.46 kg of approximately equal weights of locally purchased portabella mushrooms and vitamin D-enhanced portabella mushrooms (1.25 kg vitamin D enhanced, 1.21 kg nonenhanced) obtained directly from a supplier (Dole, West Chester, PA). The mushrooms were cleaned, trimmed, and homogenized as described above, except they were processed in two batches using two separate food processors, each containing approximately half of each type of mushroom. The homogenates were poured alternately from the two processors into a large bowl, additional liquid nitrogen was added, the composite was stirred for 2 min using a stainless steel spoon, and then while the composite was maintained frozen with liquid nitrogen, it was distributed among 192 60 mL glass jars with Teflon-lined closures, with stirring to maintain homogeneity during dispensing. Subsamples were dispensed and stored as described above.

Moisture in each composite, and in aliquots taken from throughout the dispensing sequence of the control composite, was determined by vacuum drying to constant weight at 65−70 °C and 635 mmHg. These data were used to verify the homogeneity of the control composite prior to its use and to obtain the dry mass of each mushroom sample composite.

The mushroom composites were also assayed for other nutrients; folate content has been reported in a separate paper.[40]

Sterol Analysis. Reagents and standards used were as reported previously;[41] additionally, authentic ergosterol (97.2% purity) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO), and brassicasterol (99.4% purity) was obtained from Steraloids, Inc. (Newport, RI). Standards used for calibration were corrected for the stated purity.

Sterols were analyzed as trimethylsilyl ether (TMS) derivatives after alkaline saponification of the total lipid extract,[42] using capillary gas−liquid chromatography as described previously,[41] with some modifications for the analysis of ergosterol and its metabolites. Briefly, 20 mL of the total lipid extract of 1−2 g of homogenized mushroom composite was taken for gravimetric determination of total lipid,[42] and a 5 mL portion was taken for analysis of sterols by GC with flame ionization detection (FID).

Selected samples were also analyzed by GC-MS to confirm the identity of ergosterol and ergosterol metabolites. The TMS-derivatized extracts were analyzed using a Shimadzu GC-17A and a Shimadzu QP5000 single-quadrupole GC-MS (Shimadzu Scientific Instruments, Columbia, MD) with an Rtx-5MS Crossbond 5% diphenyl/95% dimethyl polysiloxane GC column (30 m × 0.25 mm × 0.25 μm df) (Restek Corp., Bellefonte, PA) utilizing splitless injection. Column inlet pressure was set at 117.4 kPa for a carrier gas flow of 0.9 mL/min and a linear velocity of 37.3 cm/s. The oven temperature program was 250 °C, ramped at 0.5 °C/min to 265 °C, and held for 25 min, for a 55 min total run time. Injector, transfer line, and detector temperatures were 300 °C, and an 8.95 min solvent delay was used. Mass spectrometer ionization energy was set at 70 eV, and spectra were scanned in the range of m/z 35−550.

For each metabolite, a mushroom sample containing the peak at a high enough concentration (at least ∼20 μg/mL) or a concentrated extract to achieve at least this concentration was selected for GC-MS. Identification was based on the presence of the expected molecular ion (M^+) and/or most of the expected ion fragments, as well as occurrence in the expected GC elution order. The work of Teichmann and coworkers[8] was used for additional reference. Retention times relative to the epicholesterol internal standard (RRT) were established from these results. Subsequently, components in other samples were identified on the basis of the RRTs, using GC-MS to confirm closely eluting peaks in individual samples, as necessary. A shorter GC column was used for GC-MS (30 vs 60 m used for GC-FID) to improve sensitivity to the lowest concentration analytes.

Vitamin D Analysis. Vitamin D was extracted and analyzed as previously described for vitamin D_3 in food samples,[43] with modifications for the analysis of vitamin D_2, at Heartland Assays, Inc. (Ames, IA). The major modification was use of [^{3}H]-vitamin D_3 instead of nonlabeled vitamin D_3 as the internal standard due to the presence of a peak in the mushrooms that coeluted with vitamin D_3 in the routine chromatographic system used. Crystalline vitamin D_2 standard was purchased from Sigma-Aldrich (St. Louis, MO). To establish the purity of the standard, a small known amount of the material was dissolved in 100% ethanol and scanned using a Beckmann DU 640 spectrophotometer, and the concentration was determined using an extinction coefficient of 19400 at 264 nm.[44]

Homogenized mushroom sample (∼2 g), was spiked with the [^{3}H]-vitamin D_3 (30 curies/mmol) (Amersham Life Sciences, Arlington Heights, IL) internal standard, saponified in methanolic KOH (250 g KOH/30 mL H_2O/470 mL methanol) for 60 min at 60 °C, and extracted three times with 1 volume of hexane/ethyl acetate (85:15 v/v). The extract was washed with water, collected, and dried under vacuum. The dried extract was resuspended in 1.0 mL of hexane/methylene chloride (90:10 v/v), applied to a hand-packed solid-phase extraction cartridge (0.5 g of 10−40 μm silica, Varian, Palo Alto, CA; part A8501, equipped with a top stainless steel frit), eluted with methylene chloride/2-propanol (99.8:0.2 v/v), and dried. The residue was resuspended in hexane/methylene chloride/alcohols (85:15:0.2 v/v/v), with the alcohols consisting of 2:1 (by volume) isopropanol/methanol, applied to an Agilent HPLC Zorbax SIL column (5 μm, 0.9 × 25 cm, Agilent Technologies, Santa Clara, CA); the vitamin D fraction was collected and dried. The resulting residue was resuspended in hexane/alcohols (99.5:0.5 v/v) and again applied to an HPLC Zorbax NH_2 column (5 μm, 0.045 × 25 cm, Agilent Technologies); the vitamin D fraction was collected, dried, and then suspended in acetonitrile/methanol (1:1 v/v). The final separation and quantitation of vitamin D_2 were achieved by HPLC using a Vydac ODS column (Vydac part 201TP54, Chrom Tech, Inc., Apple Valley, MN) with a mobile phase of acetonitrile/methylene chloride (65:35 v/v) and UV detection at 265 nm. Vitamin D_2 was baseline-resolved from vitamin D_3 using this system. Vitamin D_2 was quantified by comparison of the UV peak areas of vitamin D_2 standards that were also spiked with an equivalent amount of [^{3}H]-vitamin D_3 and unknowns using the sample to [^{3}H]-vitamin D_3 internal standard ratio.

Quality Control. The homogeneity of the mushroom CC was evaluated by measuring moisture in triplicate, by vacuum-drying triplicate subsamples (∼2 g) to a constant weight at 65−70 °C and 635 mmHg, in each of seven subsamples drawn from across the dispensing sequence of the total of 176 subsamples dispensed, and performing an analysis of variance among the subsamples. The validation described for phytosterols in a previous paper[41] was assumed to apply to the extraction of ergosterol. The GC-MS analyses described above verified the identity of the analyte peaks. A sample of the mushroom CC was included in each analytical batch of samples, and data for this material were used to monitor run-to-run precision.

Method validation for vitamin D_2 included recovery of 60 ng of vitamin D_2 plus [^{3}H]-vitamin D_3 added to 10 samples of the mushroom CC containing a similar amount (∼60 ng) of vitamin D_2 in the analytical portion, with final quantitation using the internal standard ratio method as described above. Additionally, vitamin D_2 was confirmed by analysis of the mushroom CC, both at the USDA Food Composition and Methods Development Laboratory (FCMDL) using methodology previously reported[45] and also independently by an experienced commercial laboratory.

A blinded sample of the mushroom CC was included in each assay batch of the mushroom samples for sterols and for vitamin D. Data for the mushroom CC provided an estimate of analytical uncertainty for individual composites assayed in singlicate. Prior to the sample assays, five subsamples of the mushroom CC were assayed in triplicate in separate runs, for a total of n = 15 values, to establish an expected range

Table 1. Quality Control Data for Sterols in the Mushroom Control Composite Assayed in Four Independent Analytical Batches[a]

component	mean	SD	%RSD	HORRAT[b]
ergosterol	54.7	1.38	2.5	0.81
ergosta-5,7-dienol	5.33	0.25	4.7	1.08
ergosta-7-enol	1.29	0.06	5.0	0.92
ergosta-7,22-dienol	nd[c]			
sitosterol	nd			
campesterol	1.55	0.07	4.2	0.80
stigmasterol	nd			
brassicasterol	nd			
sitostanol	nd			
campestanol	nd			
Δ^5-avenasterol	nd			

[a] SD, standard deviation; %RSD, relative standard deviation as percent of the mean. Values are in mg/100 g fresh weight. [b] Assayed standard deviation divided by expected standard deviation.[46]. [c] Not detected (< 0.4 mg/100g).

for vitamin D. For each mushroom type, the four composites were distributed among separate assays for both sterol and vitamin D assays to avoid confounding routine run-to-run analytical variability with sample variability, which could occur if all composites of each type were run in the same assay batch.

Data Analysis. Means and standard deviations were calculated using Microsoft Office Excel (Professional edition 2003; Microsoft Corp., Redmond, WA), and analysis of variance (α = 0.05) and pairwise comparison of means, using the Student−Newman−Keuls test with a 95% confidence interval, were performed using XLSTAT (version 2011.2.06; Addinsoft, New York, NY). The ratio of the relative standard deviation (RSD) of the measured mean to the expected RSD (HORRAT) for replicate analyses of the control composite was calculated as described by Horwitz et al.[46]

RESULTS

Sterols. *Quality Control.* The mean assayed moisture content of the mushroom CC was 91.57 g/100 g, with 0.09% RSD and a range of 91.44−91.69 g/100 g. The homogeneity of the composite was supported by the lack of a statistically significant difference ($p > 0.5$) between subsamples, in the context of an established interassay precision of 0.14% for an in-house mixed food control material having a similar moisture content (Phillips et al., unpublished data).

Table 1 summarizes results for ergosterol and ergosterol metabolites in the four replicate analyses of the mushroom CC assayed in separate analytical batches.

The LOD and LOQ were 0.4 and 1 mg/100 g original sample, respectively, based on the sample weights, dilutions, and instrument detection limits. The HORRAT values of 0.80−1.08 for the components above the LOQ of 1 mg/100 g indicate good interassay precision[46] and support the lack of bias in results for these analytes in mushroom samples run in separate analytical batches.

GC-MS Confirmation of Components. Representative GC-FID chromatograms for each mushroom type are shown in Figure 1. The prominent ergosterol peak in all mushroom samples was easily identified on the basis of the mass spectra and expected GC elution order. Ergosterol displayed the M^+ (m/z 468) and expected ion fragments (Table 2).

Confirmation of the ergosterol metabolites (ergosta-7,22-dienol, ergosta-5,7-dienol, and ergosta-7-enol) posed a greater challenge due to low concentrations and possible coelution with other unidentified components. Ergosta-7,22-dienol (M^+, m/z 470), ergosta-5,7-dienol (M^+, m/z 470), and ergosta-7-enol (M^+, m/z 472) were confirmed in white button and portabella mushroom samples. Several other mushroom types had less complete GC-MS spectra due to low component concentration but still displayed some of the expected ion fragments for these components at the same RRTs, suggesting the same identifications.

The RRTs of ergosta-7,22-dienol and campesterol were very close and therefore required GC-MS to distinguish. Of all the samples analyzed, only one morel sample displayed a doublet in this region (Figure 1B) at a concentration large enough for GC-MS identification of the later eluting peak. This component had the correct M^+ for campesterol (m/z 472) and most of the expected mass fragments. The mass spectrum also compared well to that of an authentic campesterol standard run under the same GC-MS conditions. The earlier eluting peak had a mass spectrum very similar to that of ergosta-7,22-dienol, but without the prominent m/z 255 and 229 characteristic of Δ^7-sterols, but instead displayed an intense m/z 129 typical of Δ^5-sterols (Figure 2). Another morel sample (Figure 1A) displayed this same doublet, but with the earlier eluting peak much larger and matching the GC-MS spectrum of the corresponding peak in the other morel sample. The later eluting peak was at a concentration too low for GC-MS analysis in that sample. In all other mushroom samples, the peak at this RRT was confirmed to be ergosta-7,22-dienol.

A peak with a RRT matching that of a brassicasterol standard was also found only in morel mushrooms and was present in all composites of morel (Figure 1A,B, peak 1). The component was confirmed as brassicasterol via comparison to the RRT and the mass spectrum of an authentic brassicasterol standard run under identical conditions (Table 2).

Several unidentified compounds were found in mushroom samples, some of which were presumptive sterols based on GC-MS analysis and characteristics of the mass spectra. Two enoki mushroom composites had a component (~6−11 mg/100 g) that eluted between brassicasterol and ergosterol (Figure 1K, peak 8) with a mass spectrum similar to that of ergosterol, suggesting it is perhaps another ergosterol metabolite, or at least a similar sterol, but it was not analyzed further. Several other mushroom samples displayed this peak but at a much lower level. A peak representing ~3−6 mg/100 g and eluting ~0.5 min before ergosterol and also with a mass spectrum similar to that of ergosterol occurred in all of the morel samples (Figure 1A,B) but was not detected in other mushrooms. Another peak eluting ~1 min before ergosterol was present in some but not all portabella mushroom samples (and at very low levels in some of the other samples), but the concentration of ~2−3 mg/100 g was insufficient for GC-MS analysis. Additionally, there was an unknown group of three peaks eluting between campesterol and ergosta-5,7-dienol in many of the GC-FID chromatograms but most prominent in the *Agaricus bisporus* mushrooms (portabella, white button, crimini); this same group was evident in the sterol GC-FID chromatogram published by Teichmann et al.[7] Although typically present at a concentration too low for GC-MS identification, analysis of the two later-eluting peaks from two *A. bisporus* samples (portabella and white button) displayed M^+ with m/z 470 and 472 (respectively) and fragmentation patterns consistent with sterols but could not be analyzed in detail due to the low concentrations.

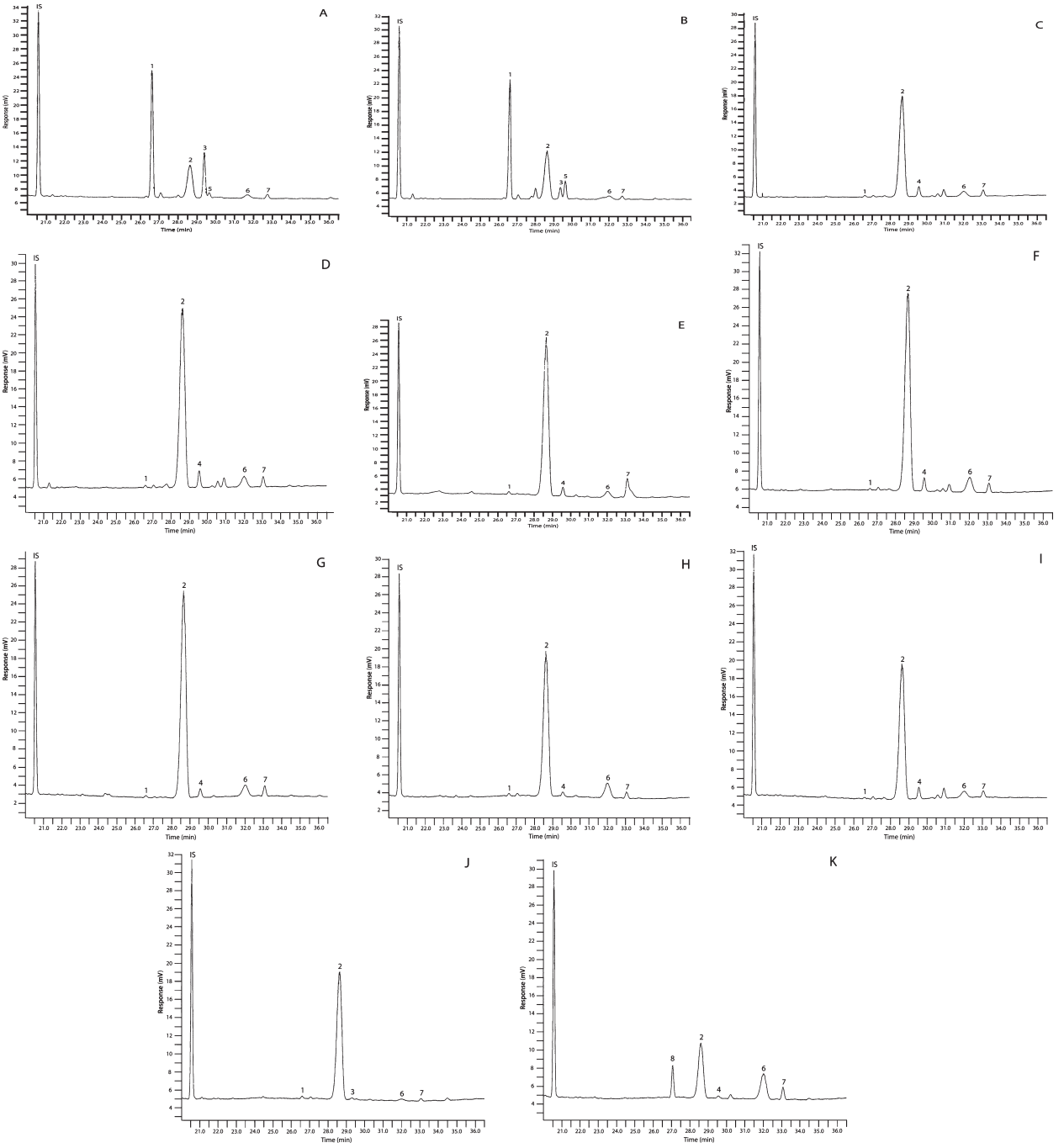


Figure 1. Representative chromatograms for each type of mushroom. Peaks: IS, internal standard (epicholesterol); 1, brassicasterol; 2, ergosterol; 3, unknown sterol; 4, ergosta-7,22-dienol; 5, campesterol; 6, ergosta-5,7-dienol; 7, ergosta-7-enol. Mushrooms (numbers in parentheses denote the composite sample as identified in Tables 3 and 4): A, morel (F1); B, morel (E2); C, portabella (3); D, portabella, UV-treated (B1); E, shiitake (2); F, white button (4); G, maitake (A1); H, oyster (1); I, crimini (2); J, chanterelle (D2); K, Enoki (G1).

Sterol Content of Mushrooms. The sterol concentrations in the mushrooms on a fresh weight basis are reported in Table 3. Shiitake and maitake mushrooms had the highest average ergosterol contents (79−85 mg/100 g), whereas morel and enoki had the lowest (26−36 mg/100 g). For most types of mushrooms there was variability among the composites for the product. Although the range in ergosterol was <10 mg/100 g among samples of white button mushrooms, it was wider for the other types, up to 50 mg/100 g in maitake. This variability meant that while average concentrations were quite disparate by mushroom type, in some cases they did not differ statistically. For example, the high means for shiitake and maitake resulted from one or two of the four composites in each case having markedly higher ergosterol concentrations (107 and 106 mg/100 g), although other composites of these species had levels similar to those in some varieties with lower average ergosterol contents.

The concentrations of sterols other than ergosterol are also shown in Table 3. Morel was the only mushroom having no ergosta-7,22-dienol, but instead a sterol at the same retention time, which was present at a relatively high concentration

Table 2. Characteristic Mass Fragments for Trimethylsilyl (TMS) Ether Sterol Derivatives of Sterols in Mushroom Samples

TMS ether	sample (Figure 1 chromatogram)	RRT[a] (min)	M^+ (m/z)	other significant fragments[b] (m/z)							
brassicasterol	morel (B)	1.293	470 {1}	455 {1}	380 {4}	365 {2}	341 {2}	255 {9}	213 {4}	129 {39}	69 {100}
ergosterol	morel (B)	1.391	468 {18}	453 {2}	378 {18}	363 {100}	337 {73}	253 {55}	211 {68}		
unknown sterol	morel (B)	1.426	470 {1}	455 {1}	386 {7}	365 {3}	343 {8}	341 {8}	253 {12}	213 {10}	129{100}
ergosta-7,22-dienol	portabella, UV-treated (D)	1.437	470 {8}	455 {8}	380 {4}	365 {8}	343 {54}	255 {100}	229 {58}	143 {33}	131 {83}
campesterol	morel (B)	1.440	472 {5}	457 {3}	382 {9}	367 {5}	343 {13}	289 {3}	255 {6}	213 {9}	129 {100}
ergosta-5,7-dienol	portabella, UV treated (D)	1.557	470 {6}	380 {14}	365 {63}	339 {54}	211 {37}	143 {100}	131 {99}	129 {54}	
ergosta-7-enol	portabella, UV treated (D)	1.608	472 {28}	457 {11}	382 {3}	367 {14}	255 {100}	229 {39}	213 {67}	143 {25}	131 {61}

[a] Retention time relative to epicholesterol (internal standard and retention reference). [b] Relative peak intensities (percent of base peak) in braces.

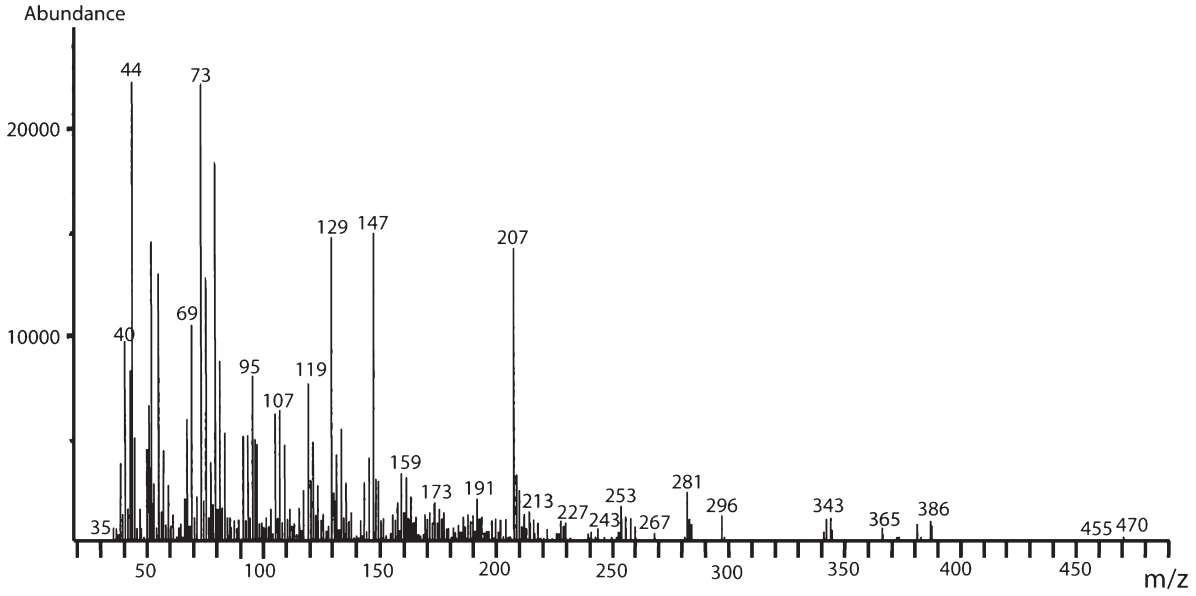


Figure 2. Mass spectrum of the prominent unidentified sterol in morel mushrooms (Table 3 and Figure 1A, peak 3).

(12.2−12.3 mg/100 g) in two of the four morel samples (Figure 1A,B and Table 4). All mushrooms except morel contained ergosta-7-enol (fungisterol), and all types contained ergosta-5,7-dienol (22,23-dihydroergosterol). It is notable that the unsaturation at C5 and C7 of the sterol B-ring of ergosta-5,7-dienol is analogous to ergosterol and 7-dehydrocholesterol from which vitamin D_2 and D_3, respectively, result upon UV exposure. Ergosta-5,7-dienol therefore would be susceptible to conversion to 22,23-dihydroergocalciferol (vitamin D_4) by the same mechanism. Enoki mushrooms had the highest concentration of ergosta-5,7-dienol (13.8−18.0 mg/100 g), whereas the mean concentration in other types ranged from an average of 4.49 mg/100 g (chanterelle) to 8.89 mg/100 g (oyster).

The relationship between ergosterol and ergosterol metabolites on a molar dry matter (DM) basis was evaluated. Composites with a low ergosterol concentration (≤30 mg/100 g DM or ≤1 mmol/100 g DM) had a higher proportion of ergosterol metabolites relative to ergosterol. Overall regression analysis showed no significant relationship between ergosterol and any of the individual components or between ergosterol and total ergosterol metabolites (data not shown). However, when the molar ratio of ergosterol to the sum of ergosterol metabolites across all composites for each mushroom species was considered, there was a linear trend for some varieties with enoki and UV-treated portabella showing a significant correlation (R^2 > 0.95) (Figure 3). Interestingly, the slope was positive (total ergosterol metabolites decreased as ergosterol increased) for all but the UV-treated portabella, which showed a negative slope (total ergosterol metabolites increased as ergosterol increased). These findings are interesting but should be considered only observational because they are based on retrospective analysis, and a specific experimental design would be needed to formally evaluate the relationship and possible covariates involved.

There were very low (<2 mg/100 g) or nondetectable (<0.4 mg/100 g fresh weight) levels of phytosterols (brassicasterol, campesterol, sitosterol, stigmasterol, sitostanol, campestanol, and Δ^5-avenasterol) in all types of mushrooms, totaling no more than 3 mg/100 g fresh weight, with a few exceptions, including some sterols that were found only in morel mushrooms (Table 4). All four morel composites contained brassicasterol (28.2−28.9 mg/100 g) at a concentration similar to that of ergosterol, whereas none of the other mushroom composites had detectable brassicasterol. Campesterol was also present at a measurable level (>0.4 mg/100 g) only in morel mushrooms (1.23−4.54 mg/100 g). Given the very close RRT of campesterol and ergosta-7,22-dienol, the GC-MS analysis (discussed above) was essential to distinguish the identity of this peak in the different types of mushrooms. Sitosterol was nondetectable in

Table 3. Vitamin D_2 and Sterol Concentrations in Mushrooms[a]

							sterols[b] (mg/100 g fresh weight)							
					vitamin D_2 (μg/100 g fresh weight)		ergosterol		ergosta-7,22-dienol		ergosta-5,7-dienol		ergosta-7-enol	
mushroom	scientific name	NDB no.[c]	composite[d]	moisture (g/100 g)	composite	mean	composite	mean	composite	mean	composite	mean	composite	mean
white button	*Agaricus bisporus*	11260	1	92.85	0.07	0.11 B	53.4	56.3 BC	1.69	1.78 A	5.97	6.03 BC	1.28	1.34 B
			2	92.81	0.09		53.2		1.73		5.79		1.37	
			3	92.35	0.06		58.7		1.89		5.86		1.24	
			4	92.47	0.23		59.8		1.82		6.49		1.47	
enoki	*Flammulina veluptipes*	11950	A1	87.68	0.04	0.14 B	33.4	35.5 DE	<1.59	<1.49 B	17.0	16.5 A	2.35	2.32 B
			A2	88.47	0.04		34.3		<1.74		18.0		2.53	
			G1	88.28	0.07		29.9		<1.64		17.0		2.98	
			1	89.30	0.40		44.6		<1.00		13.8		1.44	
shiitake	*Lentinus edodes*	11238	1	86.90	0.15	0.44 B	83.0	84.9 A	2.04	2.26 A	7.31	6.51 BC	4.63	5.03 A
			2	91.41	1.15		107		2.76		7.25		6.56	
			3	90.53	0.41		74.4		2.18		6.15		5.12	
			A1	90.11	0.03		75.4		2.05		5.34		3.79	
maitake	*Grifola frondosa*	11993	A1	88.37	0.08	28.1 A	106	79.2 AB	2.06	1.79 AB	8.90	6.34 BC	2.46	1.94 B
			A2	88.59	0.12		99.1		1.89		9.00		2.33	
			C1	92.30	63.2		55.8		1.43		3.53		1.49	
			C2	91.92	48.9		55.9		<1.65		3.92		<1.65	
oyster	*Pleurotus ostreatus*	11987	A1	89.70	0.08	0.72 B	67.6	68.0 ABC	<1.67	<1.66 AB	8.55	8.89 B	1.69	<1.7B
			1	88.77	0.13		77.3		<1.64		11.7		<1.64	
			2	90.38	2.59		70.2		<1.69		8.16		<1.69	
			3	90.54	0.07		56.7		<1.64		7.13		<1.64	
crimini	*Agaricus bisporus*	11266	1	91.92	0.03	0.06 B	66.3	61.4 ABC	2.52	2.32 A	5.25	5.92 BC	<1.67	<1.7 B
			2	91.22	0.06		68.5		2.52		6.11		<1.71	
			A1	93.08	0.08		53.3		<1.65		5.42		<1.65	
			B1	92.07	0.05		57.5		1.91		6.92		1.95	
portabella	*Agaricus bisporus*	11265	1	90.96	0.77	0.25 B	65.4	62.1 ABC	2.61	2.57 A	6.75	6.18BC	1.94	1.75B
			2	92.22	0.05		53.9		<1.63		5.45		<1.63	
			3	91.29	0.09		68.1		3.10		6.53		2.06	
			4	91.25	0.09		60.8		1.99		5.97		<1.69	
portabella, UV-treated	*Agaricus bisporus*	11998	A1	94.86	3.36	11.2 AB	48.8	51.1 CD	1.41	1.73 AB	4.57	4.70 C	1.17	1.28 B
			A2	95.12	3.64		42.2		1.03		3.94		<1.00	
			B1	94.76	16.8		52.9		2.03		5.10		1.81	
			B2	93.68	20.9		60.6		2.43		5.20		1.65	
chanterelle	*Cantharellus*	11239	D1	91.09	2.18	5.30 AB	54.9	46.3 ABC	<1.68	<1.68 AB	5.23	4.49 C	2.53	2.02 B
			D2	88.61	8.41		67.7		<1.67		3.75		<1.68	
morel	*Morchella* spp.	11240	E1	89.46	4.52	5.15 B	32.6	26.3 E	–	– C	7.13	5.79 BC	–	– C
			E2	90.38	5.43		29.8		–		5.75		–	
			F1	89.44	4.39		22.1		–		5.31		–	
			F2	89.18	6.26		20.7		–		4.98		–	

[a] –, not detected (<0.35 mg/100 g fresh weight). Analytical uncertainty ±5% for vitamin D_2 in individual composites. See Table 1 for estimates of analytical uncertainty in individual sterol values based on repeated analysis of the mushroom control composite. Means with different letters within each column differ statistically (Student–Newman–Keuls pairwise comparison, 95% confidence interval). [b] See Table 4 for sterols found only in morel mushrooms. [c] NDB number from USDA Nutrient Database for Standard Reference,[34] [d] Composite designations are arbitrary to represent the multiple composites analyzed for each type of mushroom, according to the sampling plan described in the text (locations represented by the same letter are not necessarily the same across mushroom types). Capital letters indicate that the composite was a single lot received directly from the producer (the same letter is used for the same producer across mushroom types, and different numbers from a given supplier within a mushroom type denote different lots).

all but one enoki, one morel, one crimini, two chanterelle composites that contained trace concentrations (<2 mg/100 g). Stigmasterol, sitostanol, campestanol, and Δ^5-avenasterol were not detected in any of the mushrooms.

Vitamin D. *Quality Control.* The average recovery of vitamin D_2 added to the mushroom control composite ($n = 10$) was 98.5 ± 5%. The limit of detection (LOD) was 0.1 μg/100 g in fresh mushrooms, based on the analyzed sample size (5 g) and

dx.doi.org/10.1021/jf104246z |J. Agric. Food Chem. 2011, 59, 7841–7853

Table 4. Sterols Found Only in Morel Mushrooms

	mg/100 g fresh weight		
composite[a]	campesterol	brassicasterol	unknown[b]
E1	n/r[c]	28.6	2.44
E2	4.54	28.2	2.59
F1	1.23	28.9	12.2
F2	n/r	28.6	12.3

[a] Corresponding to the composite designations in Table 3. [b] Sterol with retention time matching ergosta-7,22-dienol but confirmed by GC-MS to be another sterol (see Figure 2). [c] n/r, not reported due to insufficient resolution.

instrumental LOD. For the initial analysis of the mushroom CC in triplicate in each of five separate analytical batches, the mean vitamin D_2 content was 6.15 μg/100 g, with an average within-assay RSD of 4.1% and between-assay RSD of 3.7%. The HORRAT was 0.41, indicating excellent analytical precision.[46] The tolerance range for subsequent values was established as 5.55−6.75 μg/100 g, representing the mean ± 2 times the standard deviation of all values from the preliminary analyses. Results for the two samples of the CC run with the mushroom composite samples were 5.83 and 6.26 μg/100 g. The vitamin D_2 concentrations assayed in samples of the CC by the two other laboratories were 7.77 μg/100 g (7.4% RSD, $n = 9$) and 5.68 μg/100 g (22.6% RSD, $n = 3$).

Vitamin D Content of Mushrooms. The assayed vitamin D_2 concentrations on a fresh weight basis are shown in Table 3. *A. bisporus* mushrooms (white button, non-UV-treated portabella, and crimini), which are commonly consumed in the United States, were relatively poor sources of vitamin D, as were enoki. These species had average vitamin D_2 concentrations between 0.06 and 0.25 μg/100 g. Shiitake and oyster mushrooms had moderate vitamin D_2 contents (means: 0.44 and 0.72 μg/100 g). Among the other products (excluding the vitamin D-enhanced portabella mushrooms), morel, chanterelle, and maitake had relatively high vitamin D_2 concentrations, ranging on average from 5.15 μg/100 g in morel to 28.1 μg/100 g in maitake. Chanterelle and morel mushrooms, which are grown and harvested in the wild, had consistently higher vitamin D_2 levels (5.30 and 5.15 μg/100 g, respectively), possibly due to exposure to ambient sunlight. In some other mushroom types there was a high vitamin D concentration in just one or two composites (Table 3), possibly arising from inadvertent exposure of some of the samples to UV light during the routine retail distribution process; for example, one composite of oyster mushrooms contained 2.59 μg vitamin D_2/100 g, whereas the range for the other three composites was only 0.07−0.13 μg/100 g. The vitamin D contents of 48.9 and 63.2 μg/100 g in the two maitake mushroom samples from one supplier (Table 3, composites C1 and C2) compared to the concentration of <0.2 μg/100 g in both samples of maitake mushrooms from the other producer are likely due to UV exposure during the proprietary indoor farming process used by supplier C.[47]

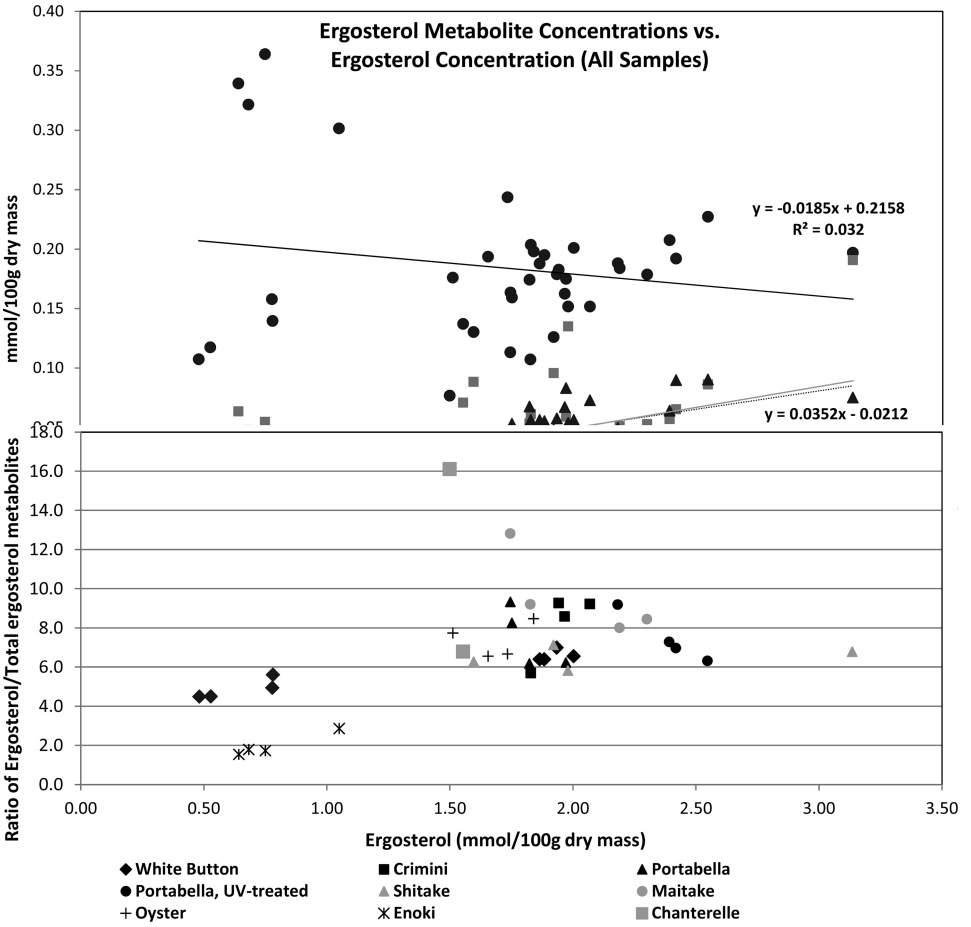
For the UV-treated portabella mushrooms, the average vitamin D_2 content was 11.2 μg/100 g. There was a statistically significant difference ($p = 0.069$) in the vitamin D_2 concentration in the samples obtained from two producers, with means of 3.50 and 18.8 μg/100 g. The divergent concentrations might have resulted from differences in the dose and wavelength of UV light used during processing.[20] Due to the limited sampling ($n = 2$) from each producer this difference cannot be generalized as a consistent divergence in the vitamin D content among all products from these producers and should be regarded as reflecting the possible variability in the vitamin D content of vitamin D-enhanced mushrooms in the retail market.

DISCUSSION

Comparison of Results to Other Literature Reports. There are limited data in the literature for the vitamin D and sterol contents of the mushrooms studied. Results from the present work are compared in Figure 4 to those of three published studies on some of the types (chanterelle, crimini, enoki, oyster, portabella, shiitake, white button)[8,17,31,32] used here. The referenced studies did not include multiple samples of each mushroom type as in the present work, but provided data for one composite sample, so that estimates of uncertainty are for analytical and not sample-to-sample variability. On the other hand, the mean values from the present work for each mushroom type shown in Figure 4 are enclosed by error bars representing the range among the separate sample composites of that mushroom. Samples analyzed by Matilla et al.[31,32] were from major producers in Finland. Samples analyzed by Teichmann et al.[8] were purchased in Sweden, with white and brown button and portabella produced in The Netherlands, oyster originating in Finland, and shiitake and chanterelle produced in Sweden. Mushrooms analyzed by Jasinghe and Perera[17] were procured in Singapore.

A low vitamin D_2 content was consistently reported for *A. bisporus* varieties (white button, crimini, non-UV-treated portabella) in all studies (Figure 4A) as well as for oyster, shiitake, and enoki. The single-sample mean vitamin D_2 content in the other literature papers fell within the range for the multiple samples analyzed in this study. However, it can be seen that in some cases, particularly oyster, the high between-sample variability meant that the vitamin D_2 concentration in an individual sample of the mushroom might deviate significantly from the existing literature values. Chanterelle mushrooms were unique. They had the highest vitamin D_2 content of the mushrooms compared, but the mean concentration (49.2 μg/100 g dry mass) and the highest concentration (73.9 μg/100 g dry mass) in any individual sample in the present study were substantially lower than the concentrations reported by Matilla et al. [33] and Teichmann et al.,[8] respectively, for *C. cibarius* (84.0 and 151 μg/100 g dry mass) and *C. tubaeformis* (194 and 301 μg/100 g dry mass). Quite possibly the growth conditions for the wild-grown chanterelle mushrooms, combined with a relatively high natural vitamin D_2 content, and the possible differences among species resulted in the greater between-sample variability. Rangel-Castro et al.[48] reported vitamin D_2 contents ranging from 0.12 to 6.30 μg/100 g dry mass in pigmented and albino stored and dried *C. cibarius* mushrooms sampled in Sweden, and there was no apparent correlation between pigmentation and vitamin D_2 level, supporting the probable high between-sample variability within this species.

Teichmann et al.[8] reported lower ergosterol concentrations in the types of mushrooms studied in common with the present work and with the reports by Mattila and co-workers[31,32] and Jasinghe and Perera,[17] except for shiitake and chanterelle (Figure 4B). However, in the absence of a common control sample it is impossible to distinguish the possible contribution of differences due to analysis versus true differences among samples of the same mushroom type from these different locations/studies. The difficulty in comparing these results highlights the



Correlation coefficients for linear regression by mushroom type:

	From Linear Trendline			
			equation	
Mushroom	R^2		m	b
Portabella, UV-treated	**0.979**		-8.07	26.67
Portabella	0.616		-11.63	28.72
White button	0.148		1.75	3.21
Crimini	0.713		14.50	-20.11
Enoki	**0.965**		3.16	-0.50
Oyster	0.068		1.72	4.45
Shitake	0.106		0.28	5.90
Maitake	0.621		-6.36	22.44
Chanterelle	n/a (n=2)			
Morel	0.726		2.79	3.09

Figure 3. Relationship between ergosterol and ergosterol metabolites in mushrooms.

need for matrix-matched interlaboratory control materials for this purpose. It should be noted that a peak present in all mushroom samples analyzed coeluted with vitamin D_3 in the chromatography system described, which is standard for vitamin D analysis,[43] and for which it is common practice to use unlabeled vitamin D_3 as the internal standard for analysis of vitamin D_2 in foods not containing D_3. However, this practice would cause an error in the quantitation of vitamin D_2 in mushrooms unless it were verified that the chromatography system separated the interfering peak from vitamin D_3. Other published results for vitamin D_2 in mushrooms should therefore be considered tentative if the studies have not included appropriate confirmation that this unknown (present at widely varying levels in all mushrooms tested) did not coelute with the internal standard under the chromatography conditions used. Tritiated vitamin D_3 was selected as the internal standard in the present study. Of course, other internal standards might be possible but the intent of this work was not an investigation of methodology, but to validate the results presented for vitamin D_2 by the method employed.

Relationship between Sterol and Vitamin D Concentrations. Ergosterol concentrations, while variable among samples of most types of mushrooms included in this study, showed a much smaller relative range both within and between species compared to vitamin D (Table 3). For ergosterol, the ratio of the highest (shiitake, 107 mg/100 g) to the lowest (morel, 20.7 mg/100 g)

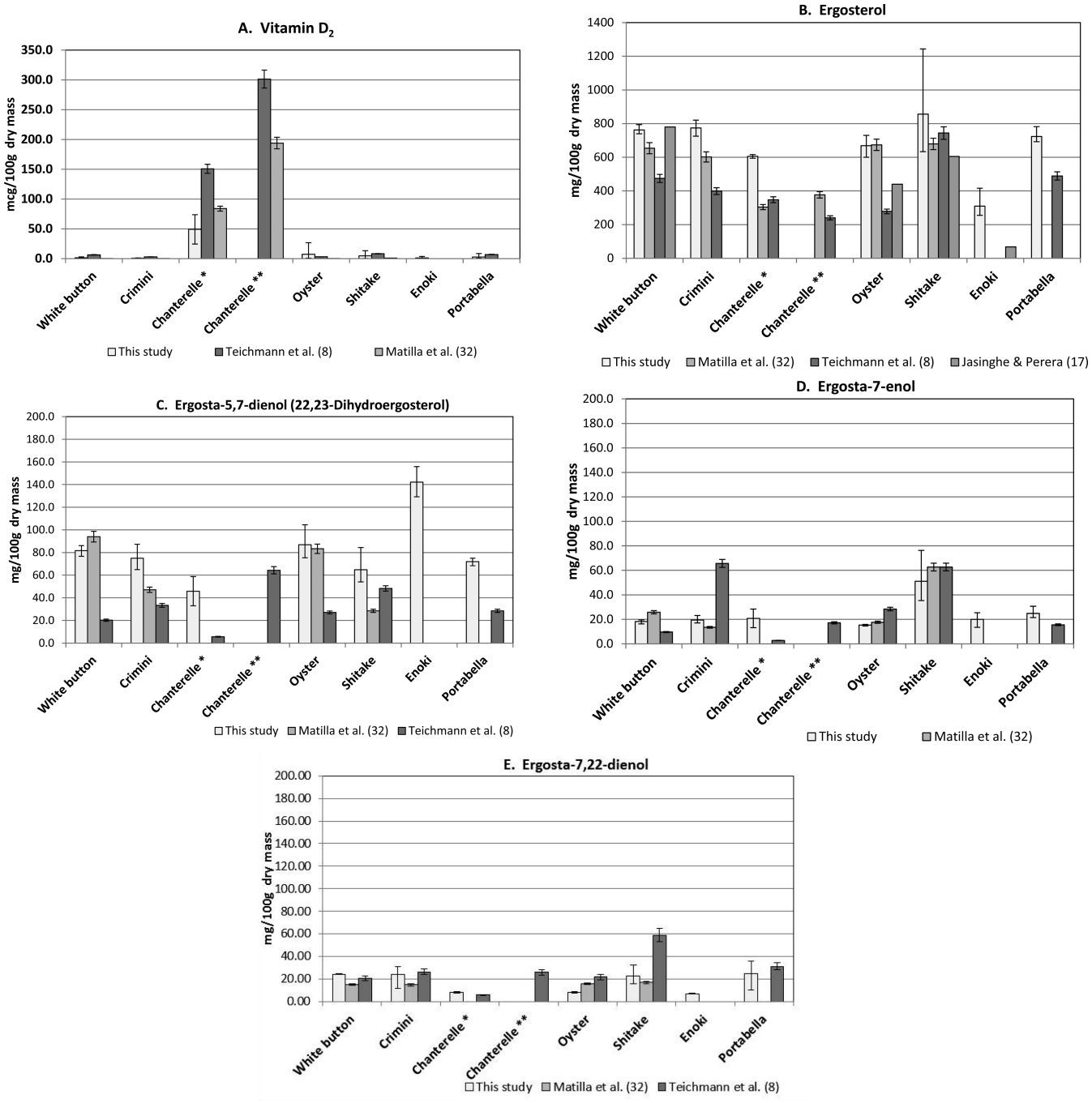


Figure 4. Comparison of results from this study (mean ± highest and lowest concentrations for $n = 4$ separate composites) to values from other studies,[8,17,31,32] for single samples of each mushroom (±5%). *, *C. cibarius*; **, *C. tubaeformis*.

average concentration was 5.2, whereas the ratio was 2107 for vitamin D (maitake/crimini, 63.2 μg/100 g/0.03 μg/100 g) among mushrooms other than those known to be UV-treated. Factors such as environmental UV exposure or other conditions during growth, storage, or handling or inherent differences in enzyme activity or metabolism are probably the primary variables affecting vitamin D_2 content in mushrooms not intentionally treated with UV light.

Potential Contribution to Vitamin D Intake. On the basis of the data presented in this study, some types of mushrooms commonly available in the U.S. retail market provide potentially nutritionally significant amounts of naturally occurring vitamin D_2. Whereas the precise biological activity of vitamin D_2 relative to D_3 has not been established, the bioavailability of vitamin D_2 from mushrooms has been demonstrated in both humans and rodents.[10,11] Vitamin D_2 is permitted by the U.S. Food and Drug Administration as a fortificant in some foods and in dietary supplements. In the following discussion, for the purpose of comparison to an RDA of 600 IU for vitamin D (equivalent to 15 μg),[5] vitamin D_2 values were calculated using the conversion factor of 40 IU/μg used for vitamin D_3. One 70 g serving (~1 cup sliced raw mushrooms) of shiitake, maitake, oyster, morel, and

chanterelle contributes, on average, 1−12.3% of the 15 μg RDA for vitamin D. The type of mushroom consumed makes a substantial difference in the amount of vitamin D_2. Whereas chanterelle and morel contain 12−12.3% of the RDA, the vitamin D contents of the more commonly consumed (in the United States) white button, crimini, and non-UV-treated portabella varieties, and also enoki, are comparatively insignificant (<0.2 μg, or <1% RDA). Vitamin D-enhanced portabella mushrooms sampled from retail markets contain significantly higher levels on average (7.8 μg, or 52.3% of the RDA per 70 g serving), but the concentrations should be expected to vary widely on a sample-to-sample basis given the variability observed in this study.

The mean ergosterol concentration in all types of mushrooms was 26−85 mg/100 g, somewhat lower as a mass percentage compared to total phytosterols in nuts and seeds (95−400 mg/100 g),[41] which are considered a rich natural source. However, the potential contribution of noncholesterol sterols, primarily ergosterol, by mushrooms is closer on a per serving basis (27−114 mg per 28.4 g serving of nuts and seeds and 19−60 mg/70 g or ∼1 cup of sliced mushrooms). Morel mushrooms also contained 29 mg brassicasterol/100 g.

The value of accurate and comprehensive food composition data and databases for non-nutrient bioactive or potentially bioactive components is becoming increasingly recognized as critical to research on the relationship between diet and health, especially in epidemiological studies and feeding trials that attempt to correlate intake of particular foods with health effects and generate hypotheses about the effective food components. Data on vitamin D content and composition of foods are important, especially given the current interest in the health effects of vitamin D.[7] Variability in the concentration of ergosterol, and more significantly vitamin D, in several mushroom types suggests the importance of analyzing these components in samples of products when the vitamin D content of the specific sample is important, such as in mushroom consumed in controlled feeding trials in which vitamin D status is evaluated. In these cases average values across the market supply could differ significantly from the concentration in any given sample. Data on variability can provide guidance in the need for such attention, considering the particular type of mushroom consumed. Additionally, the possible presence of vitamin D analogues other than D_2, arising from precursors analogous to ergosterol that were present in meaningful amounts in some mushrooms [e.g., 22,23-dihydroergosterol (ergosta-5,7-dienol) → vitamin D_4 (22,23-dihydroergocalciferol)], should be recognized, especially in UV-treated mushrooms.

Vitamin D results from this work were included in release 22 of SR,[34] adding to the body of data available to assess the vitamin D content of the U.S. diet. Results for the sterols and other nutrients in the same composites, including folate as reported separately,[40] were incorporated in release 23 of SR.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Department of Biochemistry (0308), 304 Engel Hall, Virginia Tech, Blacksburg, VA 24061. E-mail: kmpvpi@vt.edu. Phone: (540) 231-4361. Fax: (540) 231-9070.

Notes

Safety. Usual laboratory precautions should be taken when working with organic solvents (e.g., chloroform, methanol, acetonitrile, ether, hexane). Consult manufacturers' materials safety data sheets for all chemicals for specific cautionary measures and storage information.

Conflict of Interest StatementBart Minor is an employee of the U.S. Mushroom Council. Ryan Simon is an employee of Cantox Health Sciences International. Cantox has provided consulting services to the U.S. Mushroom Council within the past three years. Mary Jo Feeney is an independent consultant to the U.S. Mushroom Council. No financial or equity interests are declared.

Funding Sources

This work was supported by the USDA Agricultural Research Service as part of the National Food and Nutrient Analysis Program, including a cooperative agreement between the USDA Nutrient Data Laboratory and Virginia Tech, with funding contributed by the U.S. Mushroom Council.

ACKNOWLEDGMENT

The detailed work of Amy Rasor and Nancy Conley on coordination of sample preparation is recognized.

ABBREVIATIONS AND NOMENCLATURE

Vitamin D_3, 9,10-seco(5*Z*,7*E*)-5,7,10(19)cholestatriene-3β-ol; vitamin D_2, 9,10-seco(5*Z*,7*E*)-5,7,10(19),22-ergostatetraene-3β-ol; ergosterol, (22*E*)-(24*R*)-24-methylcholesta-5,7,22-trien-3β-ol (ergosta-5,7,22-trien-3β-ol); ergosta-5,7-dienol (22,23-dihydroergosterol), (24*R*)-24-methylcholesta-5,7-dien-3β-ol (ergosta-5,7-dien-3β-ol); ergosta-7,22-dienol, (22*E*)-(24*R*)-24-methylcholesta-7,22-dien-3β-ol (ergosta-22-dien-3β-ol); ergosta-7-enol, (24*R*)-24-methylcholesta-7-en-3β-ol (ergosta-7-en-3β-ol); brassicasterol, (22*E*)-(24*R*)-methylcholesta-5,22-dien-3β-ol (ergosta-5,22-dien-3β-ol); campesterol, (24*R*)-methylcholesta-5-en-3β-ol (ergosta-5-en-3β-ol); for detailed information on steroid nomenclature, see, Moss.[49]

REFERENCES

(1) Holick, M. F. Vitamin D deficiency. *New Engl. J. Med.* **2007**, *357*, 266–281.

(2) Bischoff-Ferrari, H. A.; Giovannucci, E.; Willett, W. C.; Dietrich, T.; Dawson-Hughes, B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am. J. Clin. Nutr.* **2006**, *84*, 18–28.

(3) Urashima, M.; Segawa, T.; Okazaki, M.; Kurihara, M.; Wada, Y.; Ida, H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am. J. Clin. Nutr.* **2010**, doi: 10.3945/ajcn.2009.29094.

(4) Zhao, X.-Y.; Feldman, D. The role of vitamin D in prostate cancer. *Steroids* **2001**, *66*, 293−300.

(5) Ross, A. C., Taylor, C. L., Yaktine, A. L., Del Valle, H. B., Eds. *Dietary Reference Intakes for Vitamin D and Calcium*; Institute of Medicine of the National Academies: Washington, DC, 2010; prepublication summary report available at http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/DRI-Values.aspx.

(6) Institute of Medicine, Food and Nutrition Board. *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*; National Academy Press: Washington, DC, 1997.

(7) Office of Dietary Supplements. *Dietary Supplement Fact Sheet: Vitamin D*; National Institutes of Health: Bethesda, MD, 2008; available online at http://ods.od.nih.gov/factsheets/vitamind.asp (accessed Oct 14, 2009).

(8) Teichmann, A.; Dutta, P. C.; Staffas, A.; Jagerstäd, M. Sterol and vitamin D_2 concentrations in cultivated and wild grown mushrooms: effect of uv radiation. *LWT − Food Sci. Technol.* **2007**, *40*, 815–822.

(9) Holick, M. F. A perspective on the beneficial effects of moderate exposure to sunlight: bone health, cancer prevention, mental health and well being. In *Sun Protection in Man*; Giacomoni, P. U., Ed.; Comprehensive Series in Photosciences; Elsevier Science: Amsterdam, The Netherlands, 2001; Vol. 3, pp 11−37.

(10) Outila, T. A.; Mattila, P. H.; Piironen, V. I.; Lamberg-Allardt, C. J. E. Bioavailability of vitamin D from wild edible mushrooms (*Cantharellus tubaeformis*) as measured with a human bioassay. *Am . J. Clin. Nutr.* **1999**, *69*, 95–98.

(11) Koyyalamudi, S. R.; Jeong, S.-C.; Song, C.-H.; Cho, K. Y.; Pang, G. Vitamin D_2 formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet irradiation. *J. Agric. Food Chem.* **2009**, *57*, 3351−3355.

(12) Holick, M. F. The cutaneous photosynthesis of previtamin D_3: a unique photoendocrine system. *J. Invest. Derm.* **1981**, *76*, 51–58.

(13) Boland, R.; Skliar, M.; Curino, A.; Milanesi, L. Vitamin D compounds in plants. *Plant Sci.* **2003**, *164*, 357–369.

(14) Horst, R. L.; Reinhardt, T. A.; Satyanarayana Reddy, G. Vitamin D metabolism. In *Vitamin D*, 2nd ed.; Pike, J. W., Glorieux, F. H., Feldman, D., Eds.; Academic Press: San Diego, CA, 2005; pp 15−36.

(15) Feldman, D.; Malloy, P. J.; Krishnan, A. V.; Balint, E.; Feldman, D. Vitamin D: biology, action, and clinical implications. In *Osteoporosis*, 3rd ed.; Marcus, R., Feldman, D., Rosen, C. J., Nelson, D., Eds.; Elsevier Academic Press: Burlington, MA, 2008; pp 317−382.

(16) Luknitskii, F. I.; Drok, Z. Zh.; Seleznev, L. G.; Meller, É. A. Photosynthesis of vitamin D2 in isopropyl alcohol. *Pharm. Chem. J.* **1975**, *10*, 1250−1253.

(17) Jasinghe, V. J.; Perera, C. O. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D_2 by UV irradiation. *Food Chem.* **2005**, *92*, 541–546.

(18) Jasinghe, V. J.; Perera, C. O. Ultraviolet irradiation: the generator of vitamin D_2 in edible mushrooms. *Food Chem.* **2006**, *95*, 638–643.

(19) Jasinghe, V. J.; Perera, C. O.; Sablani, S. S. Kinetics of the conversion of ergosterol in edible mushrooms. *J. Food Eng.* **2007**, *79*, 864–869.

(20) Roberts, J. S.; Teichert, A.; McHugh, T. H. Vitamin D_2 formation from post-harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. *J. Agric. Food Chem.* **2008**, *56*, 4541–4544.

(21) Brufau, G.; Canela, M. A.; Rafecas, M. Phytosterols: physiologic and metabolic aspects related to cholesterol-lowering properties. *Nutr. Res. (N.Y.)* **2008**, *28*, 217–225.

(22) Ling, W. H.; Jones, P. J. H. Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sci.* **1995**, *57*, 195–206.

(23) Bobek, P.; Ginter, E.; Ozdín, L. Oyster mushroom (*Pleurotus ostreatus*) accelerates the plasma clearance of low-density and high-density lipoproteins in rats. *Nutr. Res. (N.Y.)* **1993**, *13*, 885–890.

(24) Hu, S. H.; Liang, Z. C.; Chia, Y. C.; Lien, J. L.; Chen, K. S.; Lee, M. Y.; Wang, J. C. Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. *J. Agric. Food Chem.* **2006**, *54*, 2103–2010.

(25) Mori, K.; Kobayashi, C.; Tomita, T.; Inatomi, S.; Ikeda, M. Antiatherosclerotic effect of the edible mushrooms *Pleurotus eryngii* (eringi), *Grifola frondosa* (maitake), and *Hypsizygus marmoreus* (bunashimeji) in apolipoprotein E-deficient mice. *Nutr. Res. (N.Y.)* **2008**, *28*, 335–342.

(26) Subbiah, M. T.; Abplanalp, W. Ergosterol (major sterol of baker's and brewer's yeast extracts) inhibits the growth of human breast cancer cells in vitro and the potential role of its oxidation products. *Int. J. Vitam. Nutr. Res.* **2003**, *73*, 19–23.

(27) Senatore, F. Chemical constituents of some species of Agaricaceae. *Biochem. Syst. Ecol.* **1988**, *16*, 601–604.

(28) Yokokawa, H.; Mitsuhashi, T. Fatty acid and sterol compositions in mushrooms of ten species of polyporaceae. *Phytochemistry* **1980**, *19*, 2615–2618.

(29) Mallavadhani, U. V.; Sudhakar, A. V. S.; Satyanarayana, K. V. S.; Mahapatra, A.; Li, W.; van Breemen, R. B. The sterol composition of mushrooms. *Phytochemistry* **1981**, *20*, 1349–1351.

(30) 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.

(31) Mattila, P.; Könkö, K.; Eurola, M.; Pihlava, J.-M.; Astola, J.; Vahteristo, L.; Hietaniemi, V.; Kumpulainen, J.; Valtonen, M.; Piironen, V. Contents of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. *J. Agric. Food Chem.* **2001**, *49*, 2343−2348.

(32) Mattila, P.; Lampi, A.-M.; Ronkainen, R.; Toivo, J.; Piironen, V. Sterol and vitamin D_2 contents in some wild and cultivated mushrooms. *Food Chem.* **2002**, *76*, 293–298.

(33) Mallavadhani, U. V.; Sudhakar, A. V. S.; Satyanarayana, K. V. S.; Mahapatra, A.; Li, W.; van Breemen, R. B. Chemical and analytical screening of some edible mushrooms. *Food Chem.* **2006**, *95*, 58–64.

(34) USDA, Agricultural Research Service, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory. *USDA National Nutrient Database for Standard Reference*; USDA: Beltsville, MD, 2009; available online at http://www.ars.usda.gov/nutrientdata.

(35) USDA, Agricultural Research Service (ARS), Beltsville Human Nutrition Research Center, Food Surveys Research Group (Beltsville, MD) and U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics (Hyattsville, MD). *What We Eat in America*; *NHANES 2007−2008*; 2010; available online at http://www.ars.usda.gov/Services/docs.htm?docid=13793 (accessed Aug 15, 2010).

(36) Haytowitz, D. B.; Pehrsson, P. R.; Holden, J. M. The National Food and Analysis Program: a decade of progress. *J. Food Compos. Anal.* **2007**, *21* (Suppl. 1), S94−S102.

(37) Pehrsson, P. R.; Haytowitz, D. B.; Holden, J. M.; Perry, C. R.; Beckler, D. G. USDA's national food and nutrient analysis program: food sampling. *J. Food Compos. Anal.* **2000**, *13*, 379–389.

(38) Perry, C. R.; Pehrsson, P. R.; Holden, J. A revised sampling plan for obtaining food products for nutrient analysis for the USDA national nutrient database. *Proceedings, American Statistical Association*, 2003, [serial online] available from American Statistical Association (http://www.amstat.org/Sections/Srms/Proceedings/y2003f.html (accessed March 18, 2010).

(39) Trainer, D.; Pehrsson, P. R.; Haytowitz, D. B.; Holden, J. M.; Phillips, K. M.; Rasor, A. S.; Conley, N. A. Development of sample handling procedures for foods under USDA's National Food and Nutrient Analysis Program. *J. Food Compos. Anal.* **2010**, *23*, 843–851.

(40) Phillips, K. M.; Ruggio, D. M.; Haytowitz, D. H. Folate composition of ten types of mushrooms determined by liquid chromatography−mass spectrometry. *Food Chem.* **2011**in press.

(41) Phillips, K. M.; Ruggio, D. M.; Ashraf-Khorassani, M. Phytosterol composition of nuts and seeds commonly consumed in the United States. *J. Agric. Food Chem.* **2005**, *53*, 9436–9445.

(42) Phillips, K. M.; Ruggio, D. M.; Amanna, K. R. Extended validation of a simplified extraction and gravimetric determination of total fat to selected foods. *J. Food Lipids* **2008**, *15*, 309−325.

(43) Phillips, K. M.; Byrdwell, W. C.; Exler, J.; Harnly, J.; Holden, J. M.; Holick, M. F.; Hollis, B. W.; Horst, R. L.; Lemar, L. E.; Patterson, K. Y.; Tarrago-Trani, M. T.; Wolf, W. R. Development and validation of control materials for the measurement of vitamin D_3 in selected U.S. foods. *J. Food Compos. Anal.* **2008**, *21*, 527–534.

(44) Norman, A. W. In *Vitamin D: The Calcium Homeostatic Steroid Hormone*; Academic Press: New York, 1979.

(45) Byrdwell, W. C. Comparison of analysis of vitamin D_3 in foods using ultraviolet and mass spectrometric detection. *J. Agric. Food Chem.* **2009**, *57*, 2135–2146.

(46) Horwitz, W.; Kamps, L. R.; Boyer, D. W. Quality assurance in the analysis of foods for trace constituents. *J. Assoc. Off. Anal. Chem. Int.* **1980**, *63*, 1344–1354.

(47) San Diego Gas and Electric. Fickle fungi flourish in energy-sparing indoor farm. *Progress Through Design* **2009** (Summer), 2−3

(48) Rangel-Castro, J. I.; Staffas, A.; Danell, E. The ergocalciferol content of dried pigmented and albino *Cantharellus cibarius* fruit bodies. *Mycol. Res.* **2002**, *106*, 70−73.

(49) Moss, G. P. Nomenclature of steroids. *Pure Appl. Chem.* **1989**, *61*, 1783–1822.