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RESEARCH ARTICLE

Previtamin D_2, vitamin D_2, and vitamin D_4 amounts in different mushroom species irradiated with ultraviolet (UV) light and occurrence of structurally related photoproducts

Katrin Sommer | **Marissa Hillinger** | **Walter Vetter**

Department of Food Chemistry (170b), University of Hohenheim, Stuttgart, Germany

Correspondence
Walter Vetter, Department of Food Chemistry (170b), University of Hohenheim, D-70593 Stuttgart, Germany.
Email: walter.vetter@uni-hohenheim.de

Abstract

Mushrooms are rich in ergosterol and ergosta-5,7-dienol, which can be partly converted into vitamin D_2 and D_4 through ultraviolet (UV) light exposure. Typically, mushrooms have very low vitamin D contents, but it can be increased by UV irradiation. This process generates additional photoisomers scarcely studied in mushrooms due to analytical challenges. Here, we developed a new solid phase extraction (SPE) method to separate vitamin D_2, vitamin D_4, and other tri- and pentacyclic photoisomers from the much higher abundant ergosterol. Subsequent GC/MS analysis enabled the detection of ten photoisomers in eight UV-treated mushroom species, including vitamin D_2 (previtamin D_2, tachysterol$_2$, two suprasterol$_2$ and *trans*-vitamin D_2 isomers) and vitamin D_4 (previtamin D_4). Quantitated vitamin D_2 contents of 10–540 µg/100 g dry weight agreed well with the sparse literature data available for the investigated mushroom species. In addition, previtamin D_2 (nd–1950 µg/100 g dry weight) and vitamin D_4 (10–140 µg/100 g dw) were quantified in the samples. The content and photoproduct compositions varied considerably between different mushroom species.

Practical applications: The novel SPE method can be applied to study the vitamin D and photoisomer content of mushrooms.

KEYWORDS
fungi, gas chromatography, GC/MS, photoisomer, UV irradiation, vitamin D

1 | INTRODUCTION

Vitamin D is an essential lipophilic micronutrient in human nutrition, and there have been increasing reports of deficiencies in different populations.[1] The recommended daily requirements of adult humans are 10–20 µg vitamin D which is typically expressed in International Units (IU), where 1 µg (10–20 µg) vitamin D corresponds to 40 IU (400–800 IU).[2] Vitamin D is endogenously produced in the human skin upon sunlight exposure but its availability is limited during the dark season and in regions with few sunshine hours; this necessitates additional intake of vitamin D through food.[1]

Good dietary sources of vitamin D include cod liver oil (250 µg/100 g fresh weight (fw)), fillet of fatty fish (10–30 µg/100 g fw), and beef liver (1–14 µg/100 g fw), which contain the cholesterol-derived vitamin D_3 (27 carbon atoms (C_{27}), 3 double bonds (db)).[3,4] Compared to that, vitamin D is scarcely found in nonanimal foods, and mushrooms are its primary natural source for vegans due to the presence

Abbreviations: CD, circular dichroism; ES, external standard; GC, gas chromatography; HPLC, high performance liquid chromatography; ISTD, internal standard; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; QA, quality assurance standard; SC, side chain; SIM, selected ion monitoring; SPE, solid phase extraction; TLC, thin layer chromatography; UV, ultraviolet.

of vitamin D_2 (C_{28}, 4 db) along with much lower shares of vitamin D_4 (C_{28}, 3 db).[5] However, vitamin D_2 amounts in commercial mushrooms (which are usually grown in the dark) are usually only in the range of <1–10 µg/100 g fw (with the scarce exception of up to 60 µg/100 g fw in distinct wild growing species).[6–8] Even lower amounts of typically <1 µg/100 fw were reported in the few literature reports on vitamin D_4.[9,10] Thus, typical vitamin D_2 and D_4 contents of edible mushrooms are hardly suited to cover the demand of vegans. Despite this, mushrooms are remarkably rich in vitamin D_2 and D_4 precursors, with >10 mg/100 fw ergosterol and >1 mg/100 g fw ergosta-5,7-dienol.[9,11–13] These tetracyclic provitamins are converted into vitamin D_2 and vitamin D_4 after activation by ultraviolet (UV) light.[5] Recently, purposefully UV-treated fresh button mushrooms (5–20 µg/100 g vitamin D_2) and mushroom powders (58–130 mg/100 g mushroom powders) were approved as "novel food" in the European Union (based on regulation (EU) 2015/2283).[14–16] Also, the UV irradiation of specific mushroom extracts (e.g., supercritical shiitake extracts) was found to be suited to generate high vitamin D_2 amounts in the product.[17]

Chemically, the transfer of tetracyclic ergosterol (ergosta-5,7-dienol) into vitamin D_2 (D_4) involves ring opening, leading to the tricyclic vitamin D_2 (D_4). This complex conversion process involves the tricyclic intermediate previtamin D_2 (D_4) and various other photoisomers.[18] In the following, compounds related to vitamin D_2 and derived from ergosterol will be denoted with a subscript "$_2$," such as previtamin D_2, lumisterol$_2$, tachysterol$_2$, suprasterol$_2$, and *trans*-vitamin D_2 isomers.[18,19] The same variety most likely exists for vitamin D_4 (corresponding photoisomers: lumisterol$_4$, tachysterol$_4$, suprasterol$_4$, and *trans*-vitamin D_4) but their investigation in mushrooms is scarce due to analytical challenges.[9,10,20–22] Identified problems include (i) difficulties in the chromatographic separation, (ii) the impact of the temperature, and especially (iii) the wide concentration range between the analytes. Specifically, more abundant lipid compounds like ergosterol and triacylglycerols must be separated prior to the instrumental vitamin D and photoproduct analysis to avoid matrix interferences and to meet the required sensitivity.[10,23–26]

Current official methods for vitamin D_3 determination (and its hydroxylated human metabolites in blood serum)[25,27–29] involve additional purification steps (e.g., preparative HPLC) prior to HPLC/UV, LC-MSMS, or immunoassay measurements.[7,8,25,28] Still, several problems were encountered during quantification, and results of different studies turned out to be difficult to compare.[23–25,30,31] Even with LC-MSMS, which is the current method of choice in instrumental vitamin D determination, not all isobaric coelutions could be resolved, indicating the needs for further improvements.[19,32]

The aim of this study was to determine previtamin D_2, vitamin D_2, vitamin D_4, and further isomeric photoproducts in UV-irradiated mushrooms. To address previous issues, a solid phase extraction (SPE) method was developed to separate the high abundant ergosterol and other tetracyclic compounds including ergosta-5,7-dienol from the "ring-opened" vitamin D and other tricyclic forms. Unlike previous SPE methods that mainly focused on separating native forms from more polar hydroxylated metabolites,[25,29] this approach considered the difference in molecular shape and ring condensation degree. The isomeric vitamin D forms in the two SPE fractions were subsequently silylated and analyzed by gas chromatography with mass spectrometry (GC/MS) operated in selected ion monitoring (SIM) mode.[19] This method was then applied to analyze a commercial "D_2 enriched *Agaricus bisporus* ('novel food')" sample, along with seven mushroom species (*Flammulina velutipes*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Morchella esculenta*, *Hypsizygus tessulatus*, *Hericium erinaceus*, *Auricularia auricularia-judae*), which were UV irradiated in the laboratory.

2 | MATERIALS

2.1 | Chemicals and standards

Analytical certified reference materials of vitamin D_2 (CRM ≥ 99%), external standards (ES) and quality assurance (QA) standard ph. secondary (CRM ≥ 99%), vitamin D_3 (HPLC grade ≥ 98%), ergosterol (≥ 95%) and cholesterol (≥ 99%) as well as pyridine (> 99% puriss.) were ordered from Sigma-Aldrich (Darmstadt, Germany). Lumisterol$_2$ (97%), 5,6-*trans*-vitamin D_2 (95%) and previtamin D_2 were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Tachysterol$_2$ (> 90%) was from Toronto Research Chemicals (North York, Canada). 5α-cholestane (98%) was from Acros Organics (Geel, Belgium). Methanol and *n*-hexane (both HPLC grade) were delivered by Th. Geyer (Renningen, Germany) while acetonitrile (> 99.5%) was from Bernd Kraft (Duisburg, Germany). Pellets of potassium hydroxide (> 85%) and sodium hydroxide (> 99.5%) were provided by Carl Roth (Karlsruhe, Germany). Silyl 991 (N,O-*bis*(trimethylsilyl)-trifluoroacetamide and trimethylchlorosilane (TMCS), 99:1 (v/v)) was from Macherey-Nagel (Karlsruhe, Germany). Bi-distilled water was produced in-house.

2.2 | Mushroom samples and sample preparation

The following samples were from the German retail market, that is, fresh UV-treated D_2 button mushrooms ("novel food," at least 5 µg D_2/100 g fw according to EU 2018/1001[14]), untreated white button mushrooms (*Agaricus bisporus*), and seven further mushroom species (*Flammulina velutipes*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Morchella esculenta*, *Hypsizygus tessulatus*, *Hericium erinaceus*, *Auricularia auricularia-judae*), which were UV-treated in duplicate after lyophilization and homogenization. At least five fruiting bodies were pooled, immersed in liquid nitrogen, lyophilized for 5 days and ground to fine powders using a laboratory mill. Powders of untreated mushroom species (~ 2 g) were then UV treated (Section 2.3).

Aliquots of untreated and UV-treated mushroom powders (~1 g, exactly weighed) were lipid extracted and saponified in duplicate according to Hammann et al. (2016)[12] with slight modifications. In short, lipids were cold extracted with 3 × 25 mL *n*-hexane and the combined solutions were concentrated to <2 mL in a rotary evaporator (temperature 40°C) and then adjusted to exactly 2 mL with *n*-hexane. A 1 mL aliquot was removed and saponified (55°C, 30 min)

with 1 mL ethanolic KOH (5%), liquid extracted with 3 × 1 mL *n*-hexane and again adjusted to 1 mL in a nitrogen evaporator (temperature 40°C). Aliquots of the unsaponifiable matter were (i) silylated as described previously[11] for sterol analysis (100 µL) or (ii) transferred into amber screw cap vials for SPE of vitamin D compounds (400–750 µL) (Section 2.4).

2.3 | Irradiation of standard solutions and dried mushroom powders

Two identical UV lamps (Waldmann, Villingen-Schwenningen, Germany) were placed in front of the samples with a distance of 30 cm, respectively. The overall effective wavelength emission range was 280–370 nm with λ_{max} at 310 nm (UV-B). Standard solutions of ergosterol and vitamin D_2 (1 mg mL^{-1} in ethanol, respectively) were placed in UV transmitting quartz glass vessels and irradiated for finally 60 min.[19] Mushroom powders were exactly weighed into ASAP-1 stainless steel plates (Ultra Tec, Santa Ana, CA, USA) and irradiated for 30 min.

2.4 | Solid phase extraction (SPE) and liquid-liquid extraction (LLE) of saponified mushroom powders, external standard mixes, and irradiated standard solutions

SPE cartridges (Strata C18-E, 55 µm silica-based, 500 mg, 3 mL tubes; Phenomenex, Aschaffenburg, Germany) were prewashed with 3 mL of the extractant solvent (methanol/H_2O, 95:5, v/v). Sample aliquots (vitamin D_2/D_3 and cholesterol/ergosterol standards; irradiated standard solutions and the unsaponifiable matter of the mushroom powders) were evaporated to dryness (nitrogen stream, 40°C), redissolved in 200 µL methanol/H_2O (95:5, v/v) and placed on the SPE column. Samples were eluted with 10 mL methanol/H_2O, 95:5, v/v (SPE-F1, vitamin D), followed by 10 mL of the same eluent (methanol/H_2O, 95:5, v/v; SPE-F2, sterols).

SPE-F1 with the tricyclic compounds including vitamin D_2 and D_4 (10 mL) was concentrated to 5 mL, supplemented with 1 mL NaOH (pH 9), and liquid-liquid extracted (LLE) with 2 × 1 mL *n*-hexane. Combined extracts were quantitatively transferred into 4 mL amber screw cap vials and evaporated to dryness (nitrogen stream, 40°C). Silylation was performed as described previously in details.[11] Prior to GC/MS analysis, evaporated solutions of silylated vitamins were liberated from solvent (nitrogen stream, 40°C)[11] and redissolved in 25 µL internal standard solution (1.2 µg 5α-cholestane/mL *n*-hexane).

2.5 | Gas chromatography with mass spectrometry (GC/MS), calibration, and data analysis

Trimethylsilylated secondary alcohols (sterols, vitamins and other photoproducts) were measured on an Agilent 6890/5973N GC/MS system fitted with an autosampler (CTC analytics PAL system, Zwingen, Switzerland). The GC oven hosted an Optima 5-HT capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness, Macherey-Nagel, Düren, Germany), which was connected to the inlet via a deactivated guard column (1 m × 0.25 mm, Zebron Phenomenex, Aschaffenburg, Germany). The GC oven program and further parameter were reported before.[33] Measurements were conducted both in full scan mode (*m/z* 50–650) as well as in selected ion monitoring (SIM) mode. In GC/MS-SIM mode, quantifier (qualifier) ions were *m/z* 217.2 (*m/z* 372.4) for the ISTD 5α-cholestane, *m/z* 325.3 (*m/z* 456.4) for vitamin D_3, *m/z* 337.3 (*m/z* 468.4) for vitamin D_2 and previtamin D_2, as well as *m/z* 339.3 (*m/z* 470.4) for vitamin D_4 and previtamin D_4. Previtamin D_2, vitamin D_2 and vitamin D_4 were quantified by external calibration curves (each $R^2 > 0.997$) of the extracted quantifier GC/MS-SIM ion traces after ISTD correction of the individual peak areas. Contributions of the other photoproducts were calculated by the 100% method, in which individual peak areas were related to the sum of the peak areas of the SIM/full scan corrected base peaks (bp) (Table 1).

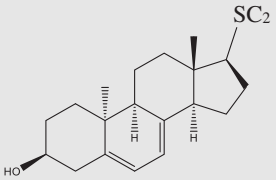
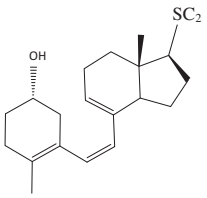
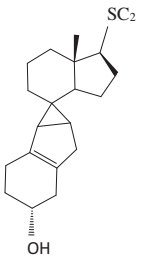
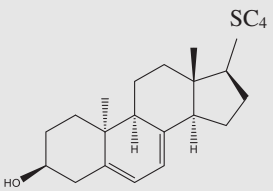
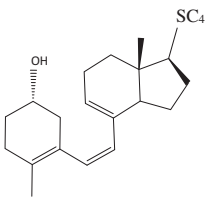
2.6 | Method validation

Method validation included determinations of the (i) *measurement precision* (via quality assurance (*QA*) standards ($n = 6$) which were directly injected into the instrument after trimethylsilylation), and comparison with (ii) *method recovery* rate of external standards (*ES*, which were submitted to SPE/LLE prior to trimethylsilylation and GC/MS analysis; $n = 6$, respectively, Figure S1). Final vitamin D_2 determinations of both, SPE/LLE separated *ES* and unfractionated *QA* solutions ($n = 6$, respectively) were quantified by an external calibration curve in the range of 0.1–100 ng μL^{-1} ($R^2 = 0.99$, 1 µL injected). Independent standard materials were used for calibration (CRM, see Section 2.1) and ES, QA standards (ph. secondary standard, see Section 2.1). Based on the external calibration data of vitamin D_2 and previtamin D_2, the *limit of detection* (LOD, $s/n = 3$) of vitamin D_2 and vitamin D_4 (no standard available) was 0.1 µg/100 g dry weight and 9.8 µg/100 g dry weight previtamins D). The practical *limit of quantification* (LOQ, $s/n = 20$) was 0.7 µg/100 g dry weight and 69 µg/100 g dry weight for D-vitamins and previtamins D, respectively.

In addition, (iii) *accuracy* of the quantitative vitamin D extraction of mushrooms was determined in triplicate ($n = 3$) with commercial UV-treated D_2 button mushrooms ("novel food") from German retail. Vitamin D_3 (cholecalciferol), which was not present in the mushrooms, served as internal standard (ISTD) for the determination of recovery (in %). The unsaponifiable matter was spiked with 1 µg of the ISTD and submitted to SPE/LLE and GC/MS analysis. Peak areas of (pyro-)vitamin D_3 in the chromatograms of the SPE/LLE extracts were compared to the peak areas in the GC/MS chromatograms of the directly injected QA standards.

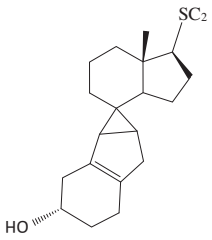
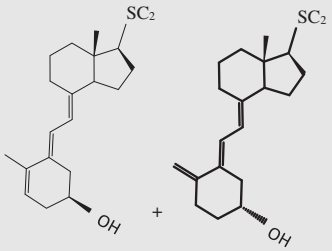
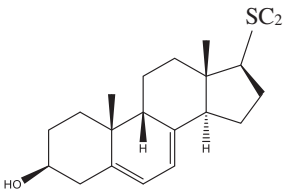
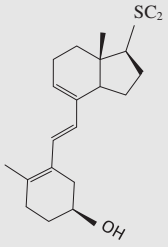
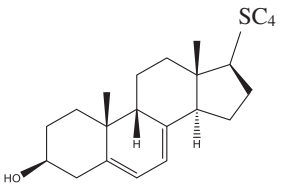
Finally, (iv) m*ethod precision* for vitamin D quantification in the UV-treated mushrooms was determined as average relative standard

TABLE 1 GC/MS data of trimethylsilylated vitamin D, photoproducts, and sterols detected in UV-treated mushrooms after SPE/LLE fractionation.

Compound	Structure	RRT	M^+ (%bp)	bp (Rf)	identification (level)/previously reported
Vitamin fraction 1					
#1 pyro-vitamin D_2*	SC_2 	1.16	468 (3%)	363 (0.06)	(A) ¡ [25–27,32] ¡¡ [22,24,50]
#2 previtamin D_2	SC_2 	1.17	468 (20%)	363 (0.05)	(A) ¡ [25–27,32] ¡¡ [22,24,50]
#3 unknown vitamin D2 isomer	n/a	1.21	468 (30%)	253 (0.06)	(C) n/a [18]
#8 suprasterol$_2$ II	SC_2 	1.22	468 (3%)	118 (0.31)	(B) ¡¡¡ [22,41,44,45,47–49]
#11 pyro-vitamin D_4*	SC_4 	1.23	470 (30%)	365 (0.06)	(B) ¡ [25–27,32]
#12 previtamin D_4	SC_4 	1.24	470 (20%)	365 (0.05)	(B) ¡ [25–27,32]

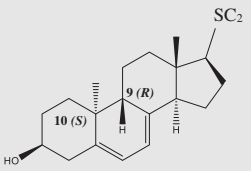
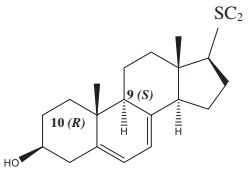
(Continues)

TABLE 1 (Continued)

Compound	Structure	RRT	M^+ (%bp)	bp (Rf)	identification (level)/previously reported
#9 suprasterol$_2$ I	SC_2 	1.25	468 (4%)	118 (0.29)	(B) iii [22,41,44,45,47,49]
#5 *trans*-vitamins D_2	SC_2 SC_2 	1.32	468 (20%)	119 (0.11)	(B) iii [41] iii [22,42–45]
#6 (isopyro) vitamin D_2*	SC_2 	1.33	468 (30%)	253 (n.d.)	(A) [22]
#7 tachysterol$_2$	SC_2 	1.38	468 (60%)	119 (0.02)	(A) i [25–27] ii [22,24,50]
#13 (isopyro) vitamin D_4*	SC_4 	1.40	470	339 (n.d.)	n/a

(Continues)

TABLE 1 (Continued)

Compound	Structure	RRT	M^+ (%bp)	bp (Rf)	identification (level)/previously reported
Sterol fraction 2					
#14 lumisterol$_2$	SC_2 	1.17	468 (15%)	363 (0.05)	(A) ¡ [25–27] ¡¡ [22,24,50]
#10 ergosterol	SC_2 	1.39	468 (30%)	363 (0.05)	(A) ¡ [16,27,38]

RRT = relative retention to ISTD 5α-cholestane. M^+ = trimethylsilylated molecular ion. bp = base peak. Rf = Response factor for SIM/full scan correction of the base peak.
*refers to pyro- and isopyro forms formed from vitamin D_2 and D_4, respectively, in the GC injector port.

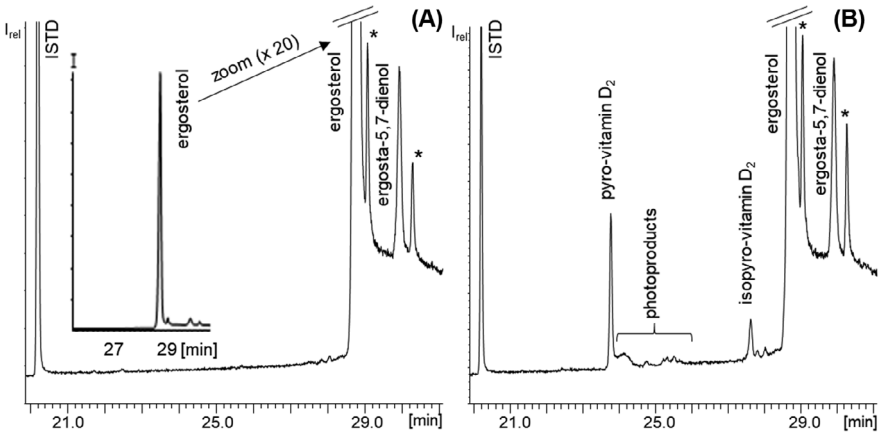


FIGURE 1 GC/MS full scan chromatograms (excerpt) of the trimethylsilylated unsaponifiable lipid fraction of *Pleurotus eryngii* (a) before and (b) after irradiation without SPE/LLE. Peaks labeled with an asterisk originate from sterols (ergosta-7,22-dienol and ergosta-7-enol), which are no vitamin D precursors. 5α-cholestane was used as the internal standard (ISTD).

deviation of the duplicate analysis ($n = 18$, Equation 1).

$$\text{Method precision } [\%] = 100 - RSD_{sample} = 100 - \left[\frac{1}{n_{batch}} \sum_{i=1}^{n_{batch}} \frac{SD_{sample}}{\bar{x}} \cdot 100\right] \quad (1)$$

3 | RESULTS AND DISCUSSION

3.1 | SPE fractionation and LLE purification of vitamin D compounds

The much higher amounts of ergosterol compared to vitamin D_2 (between three and four orders of magnitude) in the unsaponifiable lipid fraction of (nonirradiated) the mushroom samples did not allow for a direct detection of vitamin D_2 by GC/MS in the silylated sample solutions (Figure 1a). Next to ergosterol, only the much lower abundant ergosta-5,7-dienol (precursor of vitamin D_4) and two further sterols without the Δ5,7-system essential for vitamin D generation could be detected by GC/MS (Figure 1a). After UV irradiation of the mushroom powder (Section 2.3), formation of vitamin D_2 could be confirmed by the presence of pyro- and isopyro-vitamin D_2 in the GC/MS chromatogram (Figure 1b). In GC/MS injectors, vitamin D_2 is quantitatively isomerized to give a constant ratio of pyro- and isopyro-vitamin D_2[26,34] (Figure S2). *Vice versa*, neither the corresponding pyro- and isopyro products of vitamin D_4 nor other photoproducts[19] could be directly detected (Figure 1b), presumably because of the predominance of ergosterol in the irradiated mushroom samples. In the next step, ergosterol had to be removed from sample solutions by SPE (Section 2.4). Specifically, it was aimed to elute the tetracyclic ergosterol (and other intact sterols) and the tricyclic ("ring-opened") vitamin D_2 as well as related compounds into different SPE fractions. Initial attempts

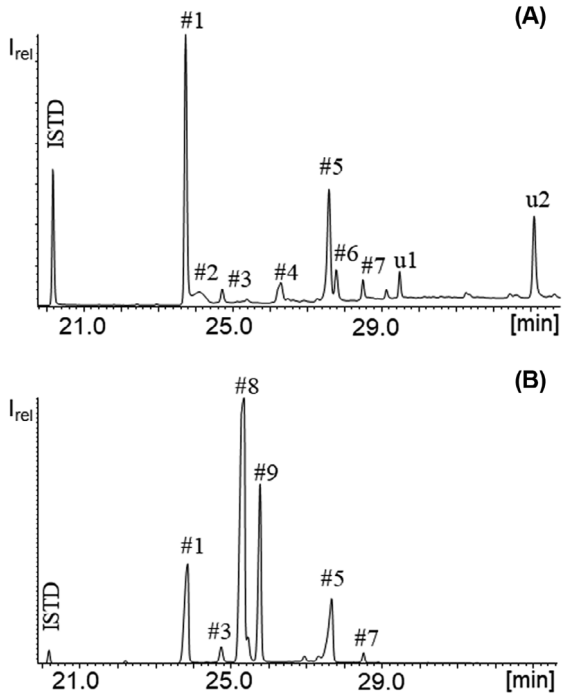


FIGURE 2 GC/MS-SIM chromatograms of SPE/LLE fraction 1 ("vitamin D fraction") of (a) UV-irradiated ergosterol and (b) UV-irradiated vitamin D_2 with tricyclic photoisomers (#1 = pyro-vitamin D_2, #2 = previtamin D_2, #3 = unknown vitamin D_2 isomer 1, #4 = unknown vitamin D_2 isomer 2, #5 = *trans*-vitamin D_2, #6 isopyro-vitamin D_2, #7 = tachysterol), and overirradiation products (#8 = suprasterol$_2$ II, #9 = suprasterol$_2$ I), and two unknown compounds (u1, u2). 5α-cholestane was used as the internal standard (ISTD).

were performed with a mix of vitamin D_2 and D_3 (both tricyclic) and the tetracyclic ergosterol and cholesterol standards at concentrations and ratios (mix ratio 1:100 and 1:1000) as previously reported in button mushrooms.[7,8,11] Using methanol as the eluent, sterols and D-vitamins eluted into the same fraction (data not shown). However, a slight reduction of the solvent strength to methanol/water (95/5, v/v) enabled the collection of the tricyclic D-vitamins with the first 10 mL (SPE-F1, Figure 2a and b) while both sterols were eluted with another 10 mL of the same solvent (SPE-F2, Figure S3a). Subsequent experiments with irradiated solutions of ergosterol and D-vitamins showed that this fractionation scheme also worked with other photoproducts. Namely, tricyclic isomers eluted with the D-vitamins into SPE-F1 while tetracyclic isomers of ergosterol were detected in SPE-F2 (see next paragraph). Since the removal of methanol/water proved to be difficult, a subsequent LLE step was carried out with the fractions. Specifically, after increasing the pH value to 9 (with NaOH), the analytes could be liquid-liquid extracted (LLE) with *n*-hexane (Section 2.4). As a further benefit, the LLE step also removed coextracted fatty acids from the SPE fractions.

Altogether, SPE-F2 featured 104 ± 9% of the sterols while D-vitamins were not detected in this "sterol fraction" with the tetracyclic compounds. Similarly, in SPE-F1 (tricyclic "vitamin D fraction") vitamin D_2 showed a *method recovery* rate of 104 ± 3% ($n = 6$), which perfectly coincided with the determined *measurement precision* of 104 ± 7%. Also, the tricyclic "vitamin D fraction" was free of tetracyclic sterols when standards were applied.

Subsequently, the method was tested in duplicate with 18 mushroom samples (nine untreated and nine UV-treated mushroom samples). In agreement with standard mixtures, D-vitamins and also previtamin D_2 were detected in SPE-F1. *Method precision* in mushrooms was determined to be 94 ± 6% for vitamin D_2, 92 ± 7% for previtamin D_2 and 87 ± 16% for vitamin D_4.

However, small shares of ergosterol (<2%) were detected in the tricyclic "vitamin D" SPE-F1 of mushroom samples (Figure 3a and b, peak #10). The slightly faster elution of ergosterol in mushroom extracts was most likely caused by matrix compounds. Although broken-through ergosterol in SPE-F1 still produced a prominent peak in the GC/MS chromatogram, it did barely hamper the evaluation of SPE-F1 (Figure 3b). On the contrary, this small share of ergosterol served us as a quality control measure because its presence indirectly verified the quantitative targeting of the earlier eluting vitamin D compounds into the anticipated SPE-F1 (traces of vitamin D compounds would have been undetectable in SPE-F2 given the high amount of ergosterols). For this reason, SPE-F2 was not evaluated in the following.

Subsequent spiking experiments with vitamin D_3 in vitamin D_2 button mushrooms ("novel food") from German retail resulted in a recovery rate of 86 ± 1% ($n = 3$) in SPE-F1 of the D-vitamins. External quantification of vitamin D_2 resulted in 19 ± 1 µg/100 g fresh weight, which is in accordance with the legal requirements (5–20 µg D_2/100 g fresh weight) of the novel food implementing regulation (EU 2018/1001 based on EU 2015/2283).[14] Hence, the present SPE method was deemed suitable for the analysis of vitamin D and photoproducts in irradiated mushroom samples.

3.2 | Inspection of SPE-F1 (tricyclic "vitamin D" fraction) of irradiated standards and dried mushroom powders

In agreement with our recent study,[19] 10 isomers were detected in UV-irradiated solutions of ergosterol and vitamin D_2 standards (Table S1, #1-#5, #7-#10, #14) and all but ergosterol (#10) and lumisterol$_2$ (#14) were eluted in SPE-F1 (vitamin D fraction) (Figure 2). As anticipated, the removal of the bulk of ergosterol and lumisterol$_2$ simplified the GC/MS-SIM chromatograms of UV-treated standards (Figure 2) and allowed to eliminate the two previously reported coelutions in GC (i.e., previtamin D_2 with lumisterol$_2$ and tachysterol$_2$ with ergosterol) (Table 1).[19] These findings underlined the value of the present SPE separation in vitamin D analysis.

Surprisingly, GC/MS analysis of a pure previtamin D_2 (#2) standard resulted in the partial formation of pyro- (#1) and isopyro-vitamin D_2 (#6) in the GC injector. Accordingly, presence of previtamin D_2 (#2) in mushroom samples will cause an overdetermination of vitamin D_2 (#1) when GC/MS is used for quantification. However, our calibration ($R^2 = 0.99$: $y = 7 \cdot 10^{-6} x^2 + 0.0021 x - 0.01$, Figure S4a) showed that a

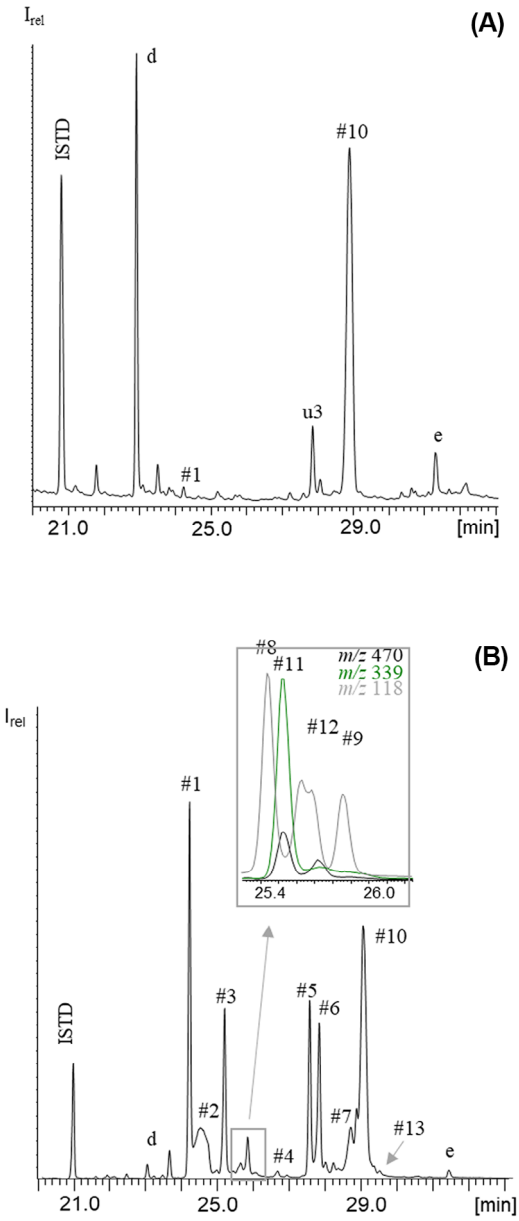


FIGURE 3 Representative silylated GC/MS-SIM chromatograms of SPE/LLE fraction 1 ("vitamin D fraction") of (a) untreated *Pleurotus eryngii* and (b) UV-treated *Pleurotus eryngii*. GC/MS-SIM chromatogram (a) only showed low shares of broken-through amounts of ergosterol (#10), traces of vitamin D_2 (#1), ergosta-3,5,7,9(11),22-pentaene (peak d), and 5,7,9(11),22-dehydroergosterol (peak e). GC/MS-SIM chromatogram (b) shows the presence of vitamin D_2 (#1) and several photoisomers (#2–#9, #11–#13). The insert in (b) shows extracted ion chromatograms (EIC) *m/z* 470 and *m/z* 339 for the detection of silylated vitamin D_4 (#11) and previtamin D_4 (#12). EIC *m/z* 118 indicated the presence of silylated suprasterol$_2$ isomers (#8, #9).

reproducible amount of previtamin D_2 (#2) underwent isomerization in dependence of the injected amount. More precisely, the share of isomerized vitamin D_2 originating from previtamin D_2 followed a polynomial regression in the range of 5–160 ng ($R^2 = 0.997$: $y = 0.04\,x^2 + 1.1\,x + 7.1$, Figure S4b). Hence, an overdetermination of vitamin D_2 could be circumvented in the present study by the determination of previtamin D_2 via peak #2 in the GC/MS chromatogram followed by subtracting the corresponding peak area from the pyro peak of vitamin D_2.

In addition to the eight isomers detected in UV-irradiated standards (#1-#5, #7-#9, Table S1), SPE-F1 ("vitamin fraction") of the *Pleurotus eryngii* sample (Figure 3b and Table 1) also featured pyro- and isopyro-vitamin D_4 (#11 and #13), previtamin D_4 (peak #12) as well as the tentatively identified ergosta-3,5,7,9(11),22-pentaene (peak d) and 5,7,9(11),22-dehydroergosterol (e)[35–39] (Figure 3b).

Ergosta-3,5,7,9(11),22-pentaene (peak d) is likely formed from 5,7,9(11),22-dehydroergosterol (Δ9(11)-double bond in addition to ergosterol), which in turn results from ergosterol by removal of water under formation of a Δ3-double bond via Nes-Mosettig rearrangement.[38,39] Ergosta-3,5,7,9(11),22-pentaene (peak d) was previously described as a bioactive compound in different natural mushroom samples (*Acremonium sp.*, *Laetiporus sulphureus*, *Cortinarius xiphidipus*)[35–37] but not in those of the present study.

Regarding vitamin D-like photoproducts, only previtamin$_{2+4}$ (#2, #12) and vitamin D_{2+4} (#1, #11), lumisterol$_{2+4}$ (#14) and tachysterol$_{2+4}$ (#7) were reported before in the few studies on photoproducts in mushrooms beyond vitamin D_2 (*Agaricus bisporus* and *Pleurotus ostreatus*).[10,21,22] Recently characterized (over)irradiation products of vitamin D_2 (#5, #8-9) and one unknown isomer (#3)[19] were preliminarily detected in mushrooms (Section 3.3).

3.3 | Photoproduct pattern in UV-treated button mushrooms and seven mushroom species

The isomers detected in SPE-F1 of the UV-irradiated *Pleurotus eryngii* sample (Table 1) were also present in all other UV-irradiated mushroom powder samples (but not in the nonirradiated mushroom samples). However, the pattern of the photoisomers varied in dependence of the mushroom type (Figure 4). Namely, vitamin D_2 contributed with ~45–73% to the sum of all photoproducts while the share of vitamin D_4 was usually very small (< 3% with one exception of ~10%). The remaining percentages were distributed between varying amounts of pre-D-vitamins (#2, #12), the photoisomers tachysterol$_2$ (#7), and the unknown isomer (#3) as well as the (over)irradiation products (*trans*-D_2 vitamins #5, and suprasterol$_2$ II #8) (Figure 4b). Notably, previtamin D_2 (#2) and previtamin D_4 (#12) are comparably instable and rapidly converted into the corresponding D-vitamins (#1, #11) at slightly elevated temperatures like in the human body.[40] Thus, previtamin D_2 (#2) and vitamin D_2 (#1) as well as previtamin D_4 (#12) and vitamin D_4 (#11) were summed up to "*total vitamin* D_2" and "*total vitamin* D_4," respectively (Figure 4a). In all mushroom species, tshare of *total vitamin* D_4 was ≤10% whereas *total vitamin* D_2 contributed with ~50% in *Flammulina velutipes* (*FV*), *Pleurotus eryngii* (*PE*) and *Morchella esculenta* (*ME*) (Figure 4a) and up to 85% to all compounds in SPE-F1 in both UV-irradiated samples of *Agaricus bisporus* (*AB and AB*(NF), Figure 4a).

In turn, additionally to *total vitamin D* (vitamin D + previtamin D[40]) highest shares of photoproducts (~50%) were present in *Flammulina velutipes*, *Pleurotus eryngii*, and *Morchella esculenta* (*FV*, *PE*, *ME*

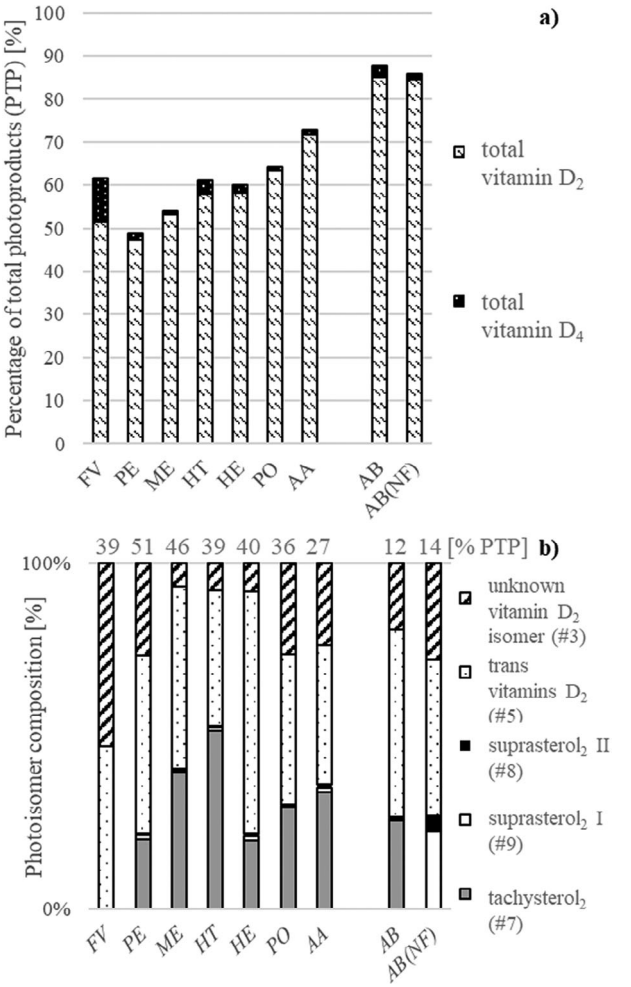


FIGURE 4 Composition of tricyclic photoproducts (percentage of total photoproducts PTP %) of UV-irradiated mushrooms with (a) previtamin and vitamin D_{2+4} were summed up to "total vitamin D," respectively and (b) composition of tricyclic photoisomers normalized to 100% and PTP next to previtamin and vitamin D_{2+4} in different mushroom species. Investigated mushroom species are *FV*: *Flammulina velutipes*, *PE*: *Pleurotus eryngii*, *ME*: *Morchella esculenta*, *HT*: *Hypsizygus tessulatus*, *HE*: *Hericum erinaceus*, *PO*: *Pleurotus ostreatus*, *AA*: *Auricularia auricularia-judae*, *AB*: *Agaricus bisporus*, *AB(NF)*: D_2 *Agaricus bisporus* ("novel food") from German retail.

in Figure 4a). In particular, *Flammulina velutipes* and *Pleurotus eryngii* showed comparably high shares of the unknown D_2 isomer #3 (*FV*, *PE* in Figure 4b). In addition, *Pleurotus eryngii* was comparably rich in *trans*-D_2 vitamins (#5) while *Flammulina velutipes* was extraordinarily high in *total vitamin* D_4 (*PE* and *FV* in Figure 4a). However, different to all other lab-irradiated mushroom species, tachysterol$_2$ (#7) could neither be detected in *Flammulina velutipes* (*FV* in Figure 4b) nor in commercial D_2 *Agaricus bisporus* (*AB*(NF) (labeled "novel food" in Figure 4b).

The photoproduct pattern of *Morchella esculenta* (*ME*) was similar to *Hypsizygus tessulatus* (*HT*), *Hericum erinaceus* (*HE*), and *Pleurotus ostreatus* (*PO*). However, *Auricularia auricularia-judae* (*AA*) differed from all other mushroom species by a very high contribution of ≥70% *total vitamin D* (Figure 4a).

Vitamin D_2 enriched *Agaricus bisporus* from German retail ("novel food," *AB*(NF)) was dominated by *total vitamin* D_2 (~85%). Also, the corresponding sample was free of tachysterol$_2$ (#7) and showed the lowest share of *trans*-D_2 vitamins (#5). By contrast, the commercial "novel food" *Agaricus bisporus* sample *AB*(NF) featured the highest contribution of the overirradiation product suprasterols$_2$ II (#8) and I (#9) (Figure 4b). Besides, previtamin D_2 was not detected which was different to the lab-irradiated *Agaricus bisporus* (*AB*) sample (and all other mushrooms). This may indicate a posttreatment conversion of the comparably unstable previtamin D_2 (#2) into vitamin D_2 (#1) during storage because the mushrooms UV treated in our laboratory were directly analyzed after the irradiation in contrast to the D_2 enriched mushrooms from German retail. In addition, lab-controlled irradiation was performed on dried mushroom powders whereas the "novel food" *Agaricus bisporus* sample was typically irradiated as fresh fruiting body containing ~91% water.[41] Further differences between the commercial "novel food" *Agaricus bisporus* sample *AB*(NF) and the lab-irradiated *Agaricus bisporus* (*AB*) sample were also observed in the relative share of the *trans*-D_2 vitamins (#5, which were higher abundant in lab-irradiated *AB*, Figure 4b). This could be due to the use of different irradiation conditions (UV source, irradiation time, source/tissue distance, temperature, etc.). However, the reported differences in the photoproduct pattern (virtually all compounds originating from the transformation of ergosterol and ergosta-5,7-dienol) of controlled lab-irradiated mushrooms suggested that species-dependent factors played an intrinsic role in vitamin D formation.

3.4 | Contents of previtamin D_2, vitamin D_2, and vitamin D_4 in UV-treated button mushrooms and seven mushroom species

Most literature data on artificially enhanced vitamin D_2 contents in mushrooms existed for *Agaricus bisporus*.[4,21,42–46] The present *Agaricus bisporus* samples showed vitamin D_2 contents of 190 ± 35 µg/100 g dw in lab-irradiated and 210 ± 15 µg/100 g dw (19 ± 1 µg/100 g fw) in the commercial "novel food" *Agaricus bisporus* sample (Table 2). Considering a water content of 9%,[41] these amounts were in accordance with the legal requirements of the novel food implementing regulation of 5–20 µg/100 g fw (EU 2018/1001 based on EU 2015/2283),[14] which corresponds with the natural contents after midday sunlight exposure.[43]

However, also much higher amounts (390–4050 µg/100 g dw) were previously determined in UV-exposed *Agaricus bisporus* samples.[4,45,46] Noteworthy, the extraordinary high vitamin D_2 contents of these *Agaricus bisporus* mushroom samples were achieved by the irradiation of (fresh or dried) mushroom slices, which is finally accompanied by an increased irradiation surface.[45,46] Next to the surface, final vitamin D_2 contents in mushrooms are also dependent on the UV exposure time, the irradiation intensity and UV source (sunlight, pulsed or continuous UV lamps, selected wavelengths, e.g., UV-A, UV-B, UV-C) along with the distance between tissue surface and emission source.[43]

TABLE 2 Vitamin D_2, vitamin D_4, and previtamin D_2 contents (μg/100 g dm) in UV-treated mushroom powders (310 nm, two UV lamps with λ_{max} = 310 nm, distance lamp/sample: 30 cm, irradiation time: 30 min).

	Vitamin D_2* (μg/100 g dw)	Vitamin D_4* (μg/100 g dw)	Previtamin D_2* (μg/100 g dw)	Previtamin D_4 (μg/100 g dw)
D_2-*Agaricus bisporus*	190 ± 35	20 ± 3	n.d.**	n.d.
Agaricus bisporus	210 ± 15	20 ± 1	1950 ± 402	n.d.
Flammulina velutipes	10 ± 1	20 ± 1	160 ± 6	n.d.
Pleurotus eryngii	540 ± 4	140 ± 2	320 ± 10	< 70
Pleurotus ostreatus	160 ± 20	30 ± 12	1710 ± 244	n.d.
Morchella esculenta	110 ± 4	10 ± 1	480 ± 13	n.d.
Auricularia auricula-judea	100 ± 3	10 ± 1	110 ± 5	n.d.
Hypsizygus tessulatus	100 ± 5	30 ± 12	1050 ± 122	< 70
Hericium erinaceus	50 ± 1	10 ± 1	100 ± 5	n.d.

*Kruskal–Wallis tests indicated no significant differences between medians of vitamin D_2, vitamin D_4 but for previtamin D_2 (**Figure S5**).
**LOD with the presented method was 0.1 μg/100 g dry weight and 10 μg/100 g dry weight for D-vitamins and previtamins D, respectively.

Vitamin D_2 contents in mushroom powders UV irradiated in our laboratory (Section 2.3) ranged by a factor of 50 from 10 ± 1 μg/100 g dry weight (dw) in *Flammulina velutipes* up to 540 ± 4 μg/100 g dw in *Pleurotus eryngii* (Table 2). While no comparable vitamin D_2 contents for UV-treated *Flammulina velutipes* were found in the current literature, Huang et al. (2015) reported the presence of up to 2.9 mg/100 vitamin D_2 in *Pleurotus eryngii* using a fourfold longer UV exposure time of 2 h (versus 30 min in our study).[47] Previous studies indicated time-depended, partly linear increase in the generation of vitamin D_2.[48] Hence, data normalized to 1 h of both studies, 1.1 and 1.5 mg vitamin D_2 h^{-1}/100 g dw, respectively, agreed well in this mushroom species.

UV irradiation of *Pleurotus ostreatus* resulted in similar vitamin D_2 contents as in *Agaricus bisporus* (160 μg/100 g dw), while the further mushroom species featured only between ~2/3 and 1/3 of this vitamin D_2 content (Table 2). Again, earlier reports on artificially enhanced vitamin D_2 contents were only found for *Pleurotus ostreatus* (3–24 mg/100 g dw)[10,49–51] and other *Hypsizigus* (1.5 mg/100 g dw) and *Auricularia* species (6 mg/100 g dw).[47]

Vitamin D_4 was present in all UV-irradiated mushroom samples and the amounts ranged between 10 μg/100 g dw in *Hericium erinaceus* and 140 μg/100 g dw in *Pleurotus eryngii*. In *Pleurotus eryngii*, the amount of vitamin D_4 was almost 30% of vitamin D_2 (Table 2) and similarly high as vitamin D_2 in *Agaricus bisporus* (Table 2). However, *Agaricus bisporus* (lab-irradiated and commercial "novel food" samples) contained 20 μg/100 g dw vitamin D_4, which represented the median level of all investigated mushroom species and ~10% of vitamin D_2 (Table 2). The vitamin D_4 contents in other mushroom species were at least one order of magnitude were lower than the vitamin D_2 amount, which is in accordance with the typically much lower amounts of its precursor ergosta-5,7-dienol compared to ergosterol.

Only very few previous studies determined vitamin D_4 levels in mushrooms,[9,10,21] and the reported quantities varied strongly even within the same mushroom species and without UV treatment. Recently, hitherto applied HPLC methods for vitamin D_2 and D_4 analysis were attributed to the varying reported quantities regarded as insufficient due to unsatisfactory detection limits and separation from coeluting isomers which for vitamin D_4.[20] Specifically, individual fresh and untreated *Morchella spp.* and *Flammulina velutipes* mushrooms contained between not detectable and ~2.3 μg/100 g fw vitamin D_4, respectively.[9] Up to date, the highest vitamin D_4 levels were determined in UV-treated portabella (up to 5.6 μg/100 g fw),[9] UV-treated *Pleurotus ostreatus* (2.3 ± 0.02 mg/100 g dw)[10] and fresh (nonirradiated) maitake mushrooms (23–35 μg/100 g fw).[9]

Literature data on previtamin D_2 contents were only available for *Pleurotus ostreatus* and *Agaricus bisporus*.[10,22] Wittig et al. (2013) determined 3.2 ± 0.1 mg previtamin D_2/100 g dw after 60-min UV-B treatment of fresh *Pleurotus ostreatus*.[10] This was 60% higher than the 2 ± 0.3 mg/100 g dw previtamin D_2 obtained after 30 min in the present study. Hence, the determined amounts in the two different studies were again almost linearly connected with irradiation time (Section 3.4, above). High previtamin D_2 contents were also found in lab-irradiated *Agaricus bisporus* (2 ± 0.4 mg/100 g dw) and *Hypsizygus tessulatus* (1 ± 0.4 mg/100 g dw) mushrooms while the amounts in the other mushrooms were two- to 10-fold lower (100 ± 5 μg/100 g dw to 480 ± 13 μg/100 g dw, Table 2). Last but not least, previtamin D_4 was only detected in UV-irradiated *Pleurotus eryngii* and *Hypsizygus tessulatus* (< LOQ of 70 μg/100 g dw), which could be partly ascribed to the ~100-fold lower GC/MS response of the previtamins compared to the corresponding vitamins (Section 2.5).

4 | CONCLUSIONS

Overall, the results of the present study underlined the importance of a full inspection of vitamin D photoisomers as various compounds were detected in UV-treated mushrooms. The presented combination of SPE fractionation and GC/MS determination enabled monitoring of the photoproduct formation and composition next to vitamin D_2 production. Differences could be outlined between different mushroom species under lab-controlled UV irradiation. In addition, notable

vitamin D_4 contents (≥10 µg/100 g dm) in seven UV-treated mushrooms and *Agaricus bisporus* indicated that this D vitamin should be routinely included in determinations of the vitamin D content of UV-treated mushrooms and considered in the calculation of International Units. Furthermore, application of the present method could simplify the evaluation of novel UV irradiation experiments (e.g., varying wavelengths, exposure times, and temperatures) with the goal to enhance vitamin D_2 contents of mushrooms and thereby the vitamin D supple of consumers. However, the GC/MS method will most likely not be able to solve all problems related to the determination of all (potential) photoproducts. Specifically, LC/MS methods tend to be more sensitive than GC/MS methods but at the dispense of the separation power. Accordingly, the present method was not developed to substitute but rather to complement currently methods in order to overcome existing problems in the thorough evaluation of photoisomers of vitamin D_2 and vitamin D_4. In this context, especially the novel SPE method could also be used and evaluated in combination with LC/MS determinations of vitamin D_2, D_4, and other tricyclic photoisomers in mushroom powers. Comprehensive methods are becoming more and more important, also for consumer protection, since new products with enhanced vitamin D_2 content (UV-treated mushrooms but also bread, prepared with UV-irradiated yeast) are entering the marked as novel food in the EU.[52]

AUTHOR CONTRIBUTIONS

Katrin Sommer: data curation; investigation; methodology; writing—original draft. **Marissa Hillinger**: investigation. **Walter Vetter**: conceptualization; supervision; resources; writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

REFERENCES

1. Wilson, L. R., Tripkovic, L., Hart, K. H., & Lanham-New, S. A. (2017). Vitamin D deficiency as a public health issue: Using vitamin D_2 or vitamin D_3 in future fortification strategies. *Proceedings of the Nutrition Society*, *76*, 392–399.
2. German Nutrition Society. (2012). New reference values for vitamin D. *Annals of Nutrition and Metabolism*, *60*, 241–246.
3. Schmid, A., & Walther, B. (2013). Natural vitamin D content in animal products. *Advances in Nutrition*, *4*, 453–462.
4. Taofiq, O., Fernandes, A., Barros, L., Barreiro, M. F., & Ferreira, I. C. F. R. (2017). UV-irradiated mushrooms as a source of vitamin D_2: A review. *Trends in Food Science & Technology*, *70*, 82–94.
5. Jäpelt, R. B., & Jakobsen, J. (2013). Vitamin D in plants: A review of occurrence, analysis, and biosynthesis. *Frontiers in Plant Science*, *4*, 136.
6. Mattila, P. H., Piironen, V. I., Uusi-Rauva, E. J., & Koivistoinen, P. E. (1994). Vitamin D contents in edible mushrooms. *Journal of Agricultural and Food Chemistry*, *42*, 2449–2453.
7. Mattila, P., Lampi, A.-M., Ronkainen, R., Toivo, J., & Piironen, V. (2002). Sterol and vitamin D_2 contents in some wild and cultivated mushrooms. *Food Chemistry*, *76*, 293–298.
8. Teichmann, A., Dutta, P. C., Staffas, A., & Jägerstad, M. (2007). Sterol and vitamin D_2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *LWT—Food Science and Technology*, *40*, 815–822.
9. Phillips, K. M., Horst, R. L., Koszewski, N. J., & Simon, R. R. (2012). Vitamin D_4 in mushrooms. *PLoS ONE*, *7*, e40702.
10. Wittig, M., Krings, U., & Berger, R. G. (2013). Single-run analysis of vitamin D photoproducts in oyster mushroom (*Pleurotus ostreatus*) after UV-B treatment. *Journal of Food Composition and Analysis*, *31*, 266–274.
11. Hammann, S., & Vetter, W. (2016). Method development for the determination of free and esterified sterols in button mushrooms (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry*, *64*, 3437–3444.
12. Hammann, S., Lehnert, K., & Vetter, W. (2016). Esterified sterols and their contribution to the total sterols in edible mushrooms. *Journal of Food Composition and Analysis*, *54*, 48–54.
13. Sommer, K., Krauß, S., & Vetter, W. (2020). Differentiation of European and Chinese truffle (*Tuber sp.*) species by means of sterol fingerprints. *Journal of Agricultural and Food Chemistry*, *68*, 14393–14401.
14. Publications Office of the European Union. (2018). Commission Implementing Regulation (EU) 2018/1011 of 17 July 2018 authorising an extension of use levels of UV-treated mushrooms as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470 (Text with EEA relevance.), 181.
15. Publications Office of the European Union. (2020). Commission Implementing Regulation (EU) 2020/1163 of 6 August 2020 authorising the placing on the market of vitamin D2 mushroom powder as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470 (Text with EEA relevance), 258.
16. Publications Office of the European Union. (2021). Commission Implementing Regulation (EU) 2021/2079 of 26 November 2021 authorising the placing on the market of vitamin D2 mushroom powder as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470 (Text with EEA relevance), 426.
17. Morales, D., Gil-Ramirez, A., Smiderle, F. R., Piris, A. J., Ruiz-Rodriguez, A., & Soler-Rivas, C. (2017). Vitamin D-enriched extracts obtained from shiitake mushrooms (*Lentinula edodes*) by supercritical fluid extraction and UV-irradiation. *Innovative Food Science and Emerging Technologies*, *41*, 330–336.
18. Jacobs, H. J. C., & Havinga, E. (2007). Photochemistry of vitamin D and its isomers and of simple trienes. *Advances in Photochemistry*, *11*, 305–373.
19. Sommer, K., Hillinger, M., Eigenmann, A., & Vetter, W. (2023). Characterization of various isomeric photoproducts of ergosterol and vitamin D_2 generated by UV irradiation. *European Food Research and Technology*, *249*, 713–726.
20. Schümmer, T., Stangl, G. I., & Wätjen, W. (2021). Safety assessment of vitamin D and its photo-isomers in UV-irradiated baker's yeast. *Foods*, *10*, 3142.
21. Keegan, R.-J. H., Lu, Z., Bogusz, J. M., Williams, J. E., & Holick, M. F. (2013). Photobiology of vitamin D in mushrooms and its bioavailability in humans. *Dermato-Endocrinology*, *5*, 165–176.
22. Kalaras, M. D., Beelman, R. B., Holick, M. F., & Elias, R. J. (2012). Generation of potentially bioactive ergosterol-derived products following

pulsed ultraviolet light exposure of mushrooms (*Agaricus bisporus*). *Food Chemistry*, *135*, 396–401.
23. Byrdwell, W. C., DeVries, J., Exler, J., Harnly, J. M., Holden, J. M., Holick, M. F., Hollis, B. W., Horst, R. L., Lada, M., Lemar, L. E., Patterson, K. Y., Philips, K. M., Tarrago-Trani, M. T., & Wolf, W. R. (2008). Analyzing vitamin D in foods and supplements: methodologic challenges. *American Journal of Clinical Nutrition*, *88*, 554S–557S.
24. Shah, I., James, R., Barker, J., Petroczi, A., & Naughton, D. P. (2011). Misleading measures in vitamin D analysis: A novel LC-MS/MS assay to account for epimers and isobars. *Nutrition Journal*, *10*, 46.
25. Roseland, J. M., Phillips, K. M., Patterson, K. Y., Pehrsson, P. R., & Taylor, C. L. (2018). Chapter 60—Vitamin D in foods: An evolution of knowledge. In D. Feldman (Ed.), *Vitamin D*, *Vol. health, disease and therapeutics* (4th ed., pp. 41–77). Academic Press.
26. Nestola, M., & Thellmann, A. (2015). Determination of vitamins D_2 and D_3 in selected food matrices by online high-performance liquid chromatography–gas chromatography–mass spectrometry (HPLC-GC-MS). *Analytical and Bioanalytical Chemistry*, *407*, 297–308.
27. Bilodeau, L., Dufresne, G., Deeks, J., Clément, J., Bertrand, G., Turcotte, S., Robichaud, A., Beraldin, F., & Fouquet, A. (2011). Determination of vitamin D_3 and 25-hydroxyvitamin D_3 in foodstuffs by HPLC UV-DAD and LC–MS/MS. *Journal of Food Composition and Analysis*, *24*, 441–448.
28. Hollis, B. W., & Frank, N. E. (1985). Solid phase extraction system for vitamin D and its major metabolites in human plasma. *Journal of Chromatography B*, *343*, 43–49.
29. Burild, A., Frandsen, H. L., & Jakobsen, J. (2014). Simultaneous quantification of vitamin D_3, 25-hydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 in human serum by LC-MS/MS. *Scandinavian Journal of Clinical and Laboratory Investigations*, *74*, 418–423.
30. Altieri, B., Cavalier, E., Bhattoa, H. P., Pérez-López, F. R., López-Baena, M. T., Pérez-Roncero, G. R., Chedraui, P., Annweiler, C., Della Casa, S., Zelzer, S., Herrmann, M., Faggiano, A., Colao, A., & Holick, M. F. (2020). Vitamin D testing: Advantages and limits of the current assays. *European Journal of Clinical Nutrition*, *74*, 231–247.
31. Stokes, C. S., Lammert, F., & Volmer, D. A. (2018). Analytical methods for quantification of vitamin D and implications for research and clinical practice. *Anticancer Research*, *38*, 1137–1144.
32. Volmer, D. A., Mendes, L. R. B. C., & Stokes, C. S. (2015). Analysis of vitamin D metabolic markers by mass spectrometry: Current techniques, limitations of the "gold standard" method, and anticipated future directions. *Mass Spectrometry Reviews*, *34*, 2–23.
33. Sommer, K., & Vetter, W. (2020). Gas chromatography with mass spectrometry detection and characterization of 27 sterols in two truffle (*Tuber*) species. *Journal of Food Composition and Analysis*, *94*, 103650.
34. Tsukida, K., & Saiki, K. (1970). Determination of vitamins D by gas-liquid chromatography. *The Journal of Vitaminology*, *16*, 293–296.
35. Torres, S., Cajas, D., Palfner, G., Astuya, A., Aballay, A., Pérez, C., Hernández, V., & Becerra, J. (2017). Steroidal composition and cytotoxic activity from fruiting body of *Cortinarius xiphidipus*. *Natural Products Research*, *31*, 473–476.
36. Ericsson, D. C. B., & Ivonne, J. N. R. (2009). Sterol composition of the macromycete fungus *Laetiporus sulphureus*. *Chemistry of Natural Compounds*, *45*, 193–196.
37. Dos Santos Dias, A. C., Couzinet-Mossion, A., Ruiz, N., Lakhdar, F., Etahiri, S., Bertrand, S., Ory, L., Roussakis, C., Pouchus, Y. F., Nazih, E.-H., & Wielgosz-Collin, G. (2019). Steroids from marine-derived fungi: Evaluation of antiproliferative and antimicrobial activities of eburicol. *Marine Drugs*, *17*, 372.
38. Nes, W. R., & Mosettig, E. (1954). The anthrasteroid rearrangement. I. The formation and proof of structure of anthraergostapentaene. *Journal of the American Chemical Society*, *76*, 3182–3186.
39. Nes, W. R. (1956). The anthrasteroid rearrangement. III. The pathway in the conversion of dehydroergosterol to anthraergostapentaene. *Journal of the American Chemical Society*, *78*, 193–198.
40. Ball, G. F. M. (1998). Vitamin D. In *Bioavailability and analysis of vitamins in foods* (pp. 163–193). New York: Springer.
41. Souci, S. W., Fachmann, W., Kraut, H., & Andersen, G. (Eds.) (2016). *Food composition and nutrition tables (Die Zusammensetzung der Lebensmittel Nährwert-Tabellen)* (8th revised and completed edition). Stuttgart, Germany: MedPharmScientific Publishers.
42. Blumfield, M., Abbott, K., Duve, E., Cassettari, T., Marshall, S., & Fayet-Moore, F. (2019). Examining the health effects and bioactive components in *Agaricus bisporus* mushrooms: A scoping review. *The Journal of Nutritional Biochemistry*, *84*, 108453.
43. Cardwell, G., Bornman, J. F., James, A. P., & Black, L. J. (2018). A review of mushrooms as a potential source of dietary vitamin D. *Nutrients*, *10*, 1498.
44. Guan, W., Zhang, J., Yan, R., Shao, S., Zhou, T., Lei, J., & Wang, Z. (2016). Effects of UV-C treatment and cold storage on ergosterol and vitamin D_2 contents in different parts of white and brown mushroom (*Agaricus bisporus*). *Food Chemistry*, *210*, 129–134.
45. Nölle, N., Argyropoulos, D., Ambacher, S., Müller, J., & Biesalski, H. K. (2017). Vitamin D_2 enrichment in mushrooms by natural or artificial UV-light during drying. *LWT—Food Science and Technology*, *85*, 400–404.
46. Urbain, P., & Jakobsen, J. (2015). Dose–response effect of sunlight on vitamin D_2 production in *Agaricus bisporus* mushrooms. *Journal of Agricultural and Food Chemistry*, *63*, 8156–8161.
47. Huang, S.-J., Lin, C.-P., & Tsai, S.-Y. (2015). Vitamin D_2 content and antioxidant properties of fruit body and mycelia of edible mushrooms by UV-B irradiation. *Journal of Food Composition and Analysis*, *42*, 38–45.
48. Jasinghe, V. J., Perera, C. O., & Sablani, S. S. (2007). Kinetics of the conversion of ergosterol in edible mushrooms. *Journal of Food Engineering*, *79*, 864–869.
49. Jasinghe, V. J., & Perera, C. O. (2005). Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D_2 by UV irradiation. *Food Chemistry*, *92*, 541–546.
50. Banlangsawan, N., & Sanoamuang, N. (2016). Effect of UV-B irradiation on contents of ergosterol, vitamin D_2, vitamin B_1 and vitamin B_2 in Thai edible mushrooms. *Chiang Mai Journal of Science*, *43*, 45–53.
51. Wu, W.-J., & Ahn, B.-Y. (2014). Statistical optimization of ultraviolet irradiate conditions for vitamin D_2 synthesis in oyster mushrooms (*Pleurotus ostreatus*) using response surface methodology. *PLOS ONE*, *9*, e95359.
52. EFSA Panel on Dietary Products, Nutrition and Allergies (NDA). (2015). Scientific opinion on the safety of UV-treated bread as novel food. *The EFSA Journal*, *13*, 4148.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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