Ultraviolet-B radiation, mushrooms, and vitamin D: From technology to bioavailability

Raquel Hidalgo-Sanz [a,1], María-Ángeles Del-Castillo-Alonso [a,1], Laura Monforte [a], Rafael Tomás-Las-Heras [a], Susana Sanz [a,*], Carmen Olarte [a], Patricia Pérez-Matute [b], María Íñiguez-Martínez [b], Alexandrina-Laura Ene [b], Javier Martínez-Abaigar [a], Encarnación Núñez-Olivera [a]

[a] *Faculty of Science and Technology, University of La Rioja, Madre de Dios 53, 26006, Logroño, La Rioja, Spain*
[b] *Center for Biomedical Research of La Rioja (CIBIR), Piqueras 98, 26006, Logroño, La Rioja, Spain*

ARTICLE INFO

ABSTRACT

We describe an efficient technology to obtain vitamin D_2-enriched mushroom powder by exposing postharvest shiitake mushrooms (*Lentinula edodes*) to UV-B radiation. UV-B exposure took place in a self-designed semi-industrial facility with the required safety measures. The highest vitamin D_2 content (1000 IU g^{-1} DM) was found when a total UV-B dose of 24 kJ m^{-2} was distributed in two different moments of the process (75% on sliced fresh mushroom and the remaining 25% on the dry powder). Similar results were obtained in king oyster (*Pleurotus eryngii*) and Portobello button (*Agaricus brunnescens*) mushrooms, indicating that the developed technology could generally be used to produce vitamin D_2 supplements from mushrooms. In addition, the same method increased the vitamin D_2 content in shiitake by-products, which could be used for animal feed. The microbiological, toxicological, and nutritional quality of the enriched mushroom preparations were confirmed to ensure a safe consumption. To determine the vitamin D bioavailability in humans, the enriched shiitake powder was encapsulated using 600 mg per capsule, which was equivalent to the minimum daily intake of vitamin D for adults. The capsules were ineffective in improving serum 25-hydroxyvitamin D status, but prevented an excessive decrease in this parameter due to winter conditions.

1. Introduction

Ultraviolet-B (UV–B: 280–315 nm) radiation is a minor fraction of the solar spectrum reaching the Earth's surface, representing only around 0.33% of the photons in the visible or photosynthetic radiations (Robson et al., 2019). At the same time, UV-B constitutes the most energetic solar wavelengths affecting organisms in the biosphere, and its level changes depending on diverse factors, such as latitude, altitude, season, hour of the day, ground albedo, cloudiness, atmospheric aerosols and stratospheric ozone. A UV-B excess is harmful for organisms and ecosystems, but damage may partially be counteracted by different protection and repair mechanisms, such as DNA repairing systems, antioxidants, and the accumulation of UV-absorbing photoprotective compounds. Thus, UV-B radiation can cause both negative and positive responses in organisms, and UV-B is nowadays rather considered as a regulator than a generic stressor. This regulation function has led to the development of diverse healthy technological innovations in agriculture and food production, including improvements in (for example) plant flavour, taste, colour, and nutritional and pharmaceutical contents (Barnes et al., 2023).

It has been widely demonstrated that UV-B radiation increases vitamin D_2 content in mushrooms, together with other healthy compounds (Cardwell, Bornman, James, & Black, 2018; Kamweru & Tindibale, 2016; Sapozhnikova, Byrdwell, Lobato, & Romig, 2014; Villares, Mateo-Vivaracho, García-Lafuente, & Guillamón, 2014). Different species have been tested in this regard, mainly *Agaricus bisporus* (Gallotti & Lavelli, 2020; Heo, Kim, Park, & Lee, 2020; Kalaras, Beelman, & Elias, 2012; Kalaras, Beelman, Holick, & Elias, 2012; Ko, Lee, Lee, & Park, 2008; Lee & Aan, 2016; Roberts, Teichert, & McHugh, 2008; Salemi et al., 2021; Urbain & Jakobsen, 2015; Urbain, Valverde, & Jakobsen,

2016), *Pleurotus ostreatus* (Gallotti & Lavelli, 2020; Hu et al., 2020), *Lentinula edodes* (Hu et al., 2020; Jasinghe & Perera, 2005; Ko et al., 2008; Morales et al., 2017), and *Pleurotus eryngii* (Singh, Gautam, & Sharma, 2021). Many different technologies have been applied in these studies, including different UV-B wavelengths, irradiances and exposure periods, as well as different parts and preparations of the mushrooms exposed to radiation. Nevertheless, there is room for improvement because few studies compare all these points together. In addition, there is scarce information on the microbiological and nutritional quality of the final product, and on the environmental aspects of these technologies, such as the potential valorization of the irradiated mushroom by-products.

In humans, UV-B radiation is a source of both negative and positive responses, because it can damage skin and eyes but also leads to the skin synthesis of vitamin D (Neale et al., 2023). The main role of vitamin D in animals is to maintain the concentration of calcium and phosphorus within the physiological range, allowing normal metabolism, neuromuscular transmission, and bone mineralization. All this is achieved through the interaction between the kidneys, bone, parathyroid gland, and the intestine. Therefore, there are multiple non-calciotropic roles of vitamin D in humans, and its deficiency is related not only to rickets and osteomalacia, but also to an increased risk of other diseases, such as diabetes mellitus, obesity, cancer, and infectious diseases including COVID-19 (Holick, 2017; Grant et al., 2020; Neill, Gill, McDonald, McRoberts, & Pourshahidi, 2023).

An adequate vitamin D intake is crucial, especially during winter, but its optimal level remains controversial. The terms deficiency or insufficiency do not lead to a clinically manifest disease, as occurs with other vitamins, because it is a hormone involved in a complex endocrine system. Nevertheless, children and adults should maintain a blood vitamin D concentration greater than 20 ng mL^{-1} to prevent osteomalacia and rickets, and this is also the adequate level for overall health in healthy individuals (NIH, 2023). Despite these recommendations, vitamin D deficiency and nutritional rickets have recently re-emerged worldwide, mainly due to changes in lifestyle generating less sun exposure for the population in general and children in particular (Holick, 2017). Risk groups for vitamin D deficiency are pregnant women, exclusively breastfed infants, older persons, people with fat absorption disorders, obese people, and people with dark skin or limited sun exposure.

Few foods naturally contain vitamin D. Fatty fish, such as salmon, tuna, and mackerel are good vitamin D sources, whereas beef liver, cheese, and egg yolk have smaller amounts (NIH, 2023). Given the relative scarcity of vitamin D in the diet, it is frequently commercialized nowadays as a food supplement or as an ingredient of fortified foods, mainly milk, margarine, breakfast cereals, and juices (Neill et al., 2023). In this context, vitamin D enrichment in UV-B-exposed mushrooms is an interesting alternative because (1) their vitamin D content is relatively high among non-animal sources, which may alleviate deficiency in humans; and (2) mushrooms could represent a primary source of vitamin D for vegetarians (Cardwell et al., 2018). The potential production of vitamin D-enriched mushrooms at a commercial scale would be particularly relevant for Spain, which occupies the seventh place in the mushroom market worldwide (Atlas Big, 2023).

The aims of the present study were (1) to develop an efficient technology to obtain vitamin D$_2$-enriched mushroom powder by exposing postharvest mushrooms to UV-B radiation; and (2) to test the effects of enriched powder on the serum levels of vitamin D in humans. To achieve these aims, we compared the effects of different UV-B exposures on three mushroom species, using different parts and preparations. In addition, we designed a pre-commercial facility for an efficient and safe irradiation of mushrooms, and we also considered environmental aspects of the process, such as the potential valorization of irradiated mushroom by-products. These advances would enhance our ability to fight against vitamin D deficiency, which nowadays represents an essential public health issue linked to several chronic diseases (Neill et al., 2023).

2. Materials and methods

2.1. Species used

We used three ecologically produced mushroom species: shiitake (*Lentinula edodes* (Berk.) Pegler), king oyster mushroom (*Pleurotus eryngii* (DC.) Quél.), and Portobello button mushroom (*Agaricus brunnescens* Peck = *Agaricus bisporus* (J.E. Lange) Imbach var. Portobello) (Fig. S1). Mushrooms were cultivated on pasteurized substrates. The ecological production processes met the requirements of CPAER (2023), on the basis of EU regulations, specifically European Union (2018). After harvest, mushrooms were packed in 2.5–3.0 kg boxes, and transported to the laboratory in refrigerated vehicles to conserve the cold chain. The material was always processed within 24 h from harvest.

2.2. Sample preparation

Once in the laboratory, the commercial parts of the three mushrooms were separated with a knife. The shiitake by-products (both stalk bases and not commercially useable individuals) were washed in a container with deionized water under constant shaking for 1 h to remove substrate residues, and dried with absorbent paper. Both the commercial parts of the three mushrooms and the shiitake by-products were cut into 3 mm thick slices with a compact food processor (Bosch MultiTalent 8, Stuttgart, Germany). The shiitake slices were dried at two different temperatures (40 and 80 °C) in a gravity convection oven (Heraeus D-6450, Hanau, Germany) until constant mass (Fig. S2), whereas the slices of king oyster and Portobello mushrooms were dried only at 40 °C. The dried slices were then ground in a mill (Culatti DFH 48, Culatti AG, Steinerberg, Switzerland) and sieved through a 1 mm sieve to obtain a homogeneous powder.

2.3. Dose-response study

A dose-response study (UV–B dose applied *vs.* vitamin D$_2$ content in mushrooms) was performed on two types of material (fresh slices and powder dried at 40 °C) of the shiitake commercial part. Five UV-B doses were used (0, 9, 12, 24, and 48 kJ m^{-2}) by applying proportional UV-B exposures between 0 and 50 min. Three different treatments were set up, with three replicates for each treatment.

- Fresh irradiation (FI), exposing fresh slices to each UV-B dose.
- Dry irradiation (DI), exposing dry powder.
- Fresh + dry irradiation (FDI), exposing fresh slices to 75% of each UV-B dose and applying the remaining 25% to the dry powder obtained from those fresh slices.

The dose-response study was also performed on the shiitake by-products and the commercial parts of king oyster and Portobello mushrooms, using only the FDI treatment. In king oyster and Portobello mushrooms, the maximum UV-B dose applied was 24 kJ m^{-2}.

The experimental design was the same for all the experiments, varying only the UV-B exposure time and, consequently, the UV-B dose applied. For each type of material (fresh slices, dry powder, and by-products), control samples (no UV-B exposure) were covered with polycarbonate filters (Macoglass S.L., Valladolid, Spain), which cut off UV radiation. For the application of the remaining UV-B doses, the material was covered with metacrylate filters (Macoglass S.L.), which allowed UV-B radiation to get through. All the samples were placed under the UV-B lamps (1.2 m-long narrow-band fluorescent tubes, TL40W/01 RS, Philips, Amsterdam, Netherlands) and, after the appropriate exposure time for each dose, the corresponding samples were covered with UV-opaque polycarbonate filters. This process was repeated until completing all the doses. In this way, all the samples of each material were exposed to the same conditions during all the process, except the varying UV-B doses received.

2.4. UV-B exposure of the samples

Samples were exposed to UV-B radiation using a self-designed and manufactured semi-industrial plant (Fig. S3) which allows to develop a technology exportable to the industry sector. The plant consisted of a conveyor belt (2.5 × 0.8 m) mounted on slotted aluminium profiles, with a brush placed at the lower side for an easy cleaning. All the materials met the FDA's (Food and Drug Administration) requirements for food use. The UV-B source was integrated in the plant and was composed by 12 1.2 m-long narrow-band fluorescent tubes (TL40W/01 RS) separated by 6.3 cm between them. The UV-B source was located at 25 cm over the belt, in a cabinet made with polycarbonate filters to guarantee the UV protection of the machine operator. UV-B treatment was performed by using metacrylate filters, which allowed UV-B radiation to get through, whereas polycarbonate filters were used for control samples. The spectral irradiances emitted by the lamps (Fig. S4) and received by the samples were measured with a spectroradiometer (Macam SR9910, Macam Photometrics Ltd., Livingston, Scotland). UV-B-treated samples received a UV-B irradiance of 16 W m^{-2}.

A stainless steel hopper with a 0.8 × 0.2 m mechanical opening and a vibrating motor was coupled to the belt for an automatic distribution of the irradiated material. Once finished the irradiation period, a photocell activated again the movement of the belt to deposit the irradiated or control samples in food bags for storage. The process was completely automated through a PLC (SIMATIC S7 1200, Siemens, Munich, Germany) with a CPU 1212C AC/DC/RLY of 75 kB (Siemens). An emergency stop sequence was implemented in the programming for safety reasons.

2.5. Vitamin D$_2$ extraction and analysis

Vitamin D$_2$ extraction and analysis were performed following AOAC (1990). For extraction, mushroom powder (0.5 g) was mixed with 10 mL of ethanol (99%), 0.5 g of ascorbic acid and 5 mL of KOH (50%). The standard vitamin D$_3$ (Sigma Chemical Co., St. Louis, MO, USA) was added (10 µg) to the sample and the extraction procedure was carried out. The solutions were put in a hot water bath (85 °C) under reflux for 30 min. After cooled to room temperature and centrifuged, the residue was extracted three times with 2 mL of water and 6 mL of n-hexane and centrifuged at 6000 g for 15 min. The pooled organic layers were washed three times with deionized water, rotary evaporated to dryness and immediately re-dissolved in 1 mL methanol:acetonitrile (75:25 v/v) and isopropyl (2:1) and filtered (PTFE, 13 mm with a pore size of 0.22 µm).

After extraction, a volume of 5 µL of filtered sample was injected into a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) equipped with an Acquity UPLC T-UV detector (Waters Corp.) and eluted through a C18 column (Acquity UPLC BEH 1.7 µM; 2.1 × 100 mm, Waters Corp.). Solvents were: A, water and B, methanol:acetonitrile (75:25 v/v), at a flow rate of 0.45 mL min^{-1}, column temperature 35 °C and UV detection at 265 nm. The gradient program employed was: 0–2 min, 50–100% B; 2–4 min, 100% B; 4–5 min, 100–50% B. A representative chromatogram is shown in Fig. S5. Vitamin D$_2$ was quantified by comparing the peak area to that of the internal standard vitamin D$_3$. The standard calibration curve was achieved plotting the ratio of the peak areas of vitamin D$_3$ and D$_2$ against the concentration of vitamin D$_2$ standard (Sigma Chemical Co.) solutions. Vitamin D$_2$ content was expressed as international units (IU) per g dry mass (DM). Vitamin D$_2$ was analysed in all the material types used in the dose-response study.

2.6. Analysis of heavy metals and pesticide residues

These analyses were performed by a certified laboratory (Kudam, Alicante, Spain) at the reception of the material, to verify that the fresh samples of the three species studied met the requirements of ecological culture and were suitable for human consumption. For heavy metals analysis, the samples were dried (105 °C for 24 h until constant mass) and digested with aqua regia in a microwave for ICP/MS analysis (Table S1). Pesticide residues were analysed by GC/MS or LC/MS (Kudam, 2023).

2.7. Microbiological analysis

For the microbiological analysis, 2 g of dry mushroom powder were homogenized in 248 mL of peptone buffer. After sample homogenization, 0.1 mL was inoculated into the selective media for each pathogen tested except for *Salmonella*, which requires prior enrichment for 24 h. All analyses were performed in duplicate. The microorganisms analysed and the respective culture media and used procedures were those recommended by ISO (2019) (Tables S2–S5). The microbiological analyses were carried out on the same materials used for vitamin D$_2$ analysis.

2.8. Nutritional analysis

The analysis of the nutritional composition of the shiitake powder was carried out by the same certified laboratory mentioned above (Kudam, Alicante, Spain). The variables analysed and respective methods were: water content by drying and weighing; proteins by Kjeldahl method; fats by Soxhlet; fatty acids by GC; carbohydrates by UV spectrophotometry; sugars by polarimetry; energy value by calculation; and salt and sodium by selective electrodes.

2.9. Encapsulation of shiitake powder

The powder obtained from shiitake commercial parts (dried at 40 °C) was encapsulated by a specialized company (Plantapol SLU, Zaragoza, Spain) to test its use as a food supplement. Each capsule, made of alimentary gelatin, contained 600 mg ± 5% of powder and capsules were packed in blisters of 10 units (Fig. S6). Vitamin D$_2$ content in the powder was measured at the moment of encapsulation and six months later to evaluate the possible degradation of vitamin D$_2$ with time.

2.10. Bioavailability study

This study was performed following the Helsinki Declaration and was approved by the Committee for Ethics in Drug Research in La Rioja (CEImLAR) (6th November 2020, reference number 449). All participants provided their written informed consent. A total of 28 healthy Caucasian individuals were recruited: 14 males and 14 females. One woman was removed from the study because she developed a serious allergy after the ingestion of the first capsule. Therefore, the final number of participants was 27: 14 males and 13 females.

Vitamin D$_2$ supplementation was carried out during 8 winter weeks between December 2020 and February 2021, to minimize body vitamin D$_2$ activation. A double-blind trial was conducted: the placebo group ingested a capsule of mushroom powder not UV-B irradiated per day during 8 weeks while the Vitamin D group ingested a capsule of mushroom powder irradiated with UV-B during the same 8 weeks (Fig. S7). The vitamin D$_2$ content was around 600 IU per capsule of irradiated powder, within the range proposed in the general recommendations for the population (600–800 IU per day: Ross et al., 2011; NIH, 2023). To confirm that the potential variations observed in our study were only due to the nutritional supplementation and not to dietary changes, all participants (both placebo and vitamin D groups) were informed not to change their dietary habits during the study period and to avoid vitamin D-supplemented foods during the four weeks prior to the start of the study. They were also encouraged to fulfil a weekly record of the consumption of foods rich in vitamin D in order to determine the possible influence of diet on vitamin D plasma levels.

Blood samples from participants were collected at the Center for Biomedical Research of La Rioja (CIBIR) at the beginning of the study, and one and two months afterwards. Serum levels of 25-hydroxyvitamin D were measured by MCIA (Microparticle Chemiluminescent

Immunoassay) by a certified laboratory (Eurofins/MEGALAB, Madrid, Spain).

2.11. Statistical analysis

The global effects of the UV-B dose and type of treatment (FI, DI, and FDI) on the vitamin D_2 content of the shiitake commercial part were tested using a two-way analysis of variance (ANOVA), once proved that the data met the assumptions of normality (Shapiro–Wilks's test) and homoscedasticity (Levene's test). In addition, one-way ANOVA was applied to test the effect of UV-B dose on the vitamin D_2 content of the shiitake by-products and the commercial parts of king oyster and Portobello mushrooms. In the case of significant differences, means were then compared by Tukey's test. Student's *t* tests were performed to analyse the effects of the drying temperature (40 *vs.* 80 °C), and the preservation period after powder encapsulation (0 *vs.* 6 months). In the bioavailability study, categorical variables were analysed using the Chi-square or Fisher's exact tests. Normal distribution of quantitative variables was checked using the Shapiro–Wilks's test. Comparisons between the two groups (placebo *vs.* vitamin D) at the beginning of the study were performed using unpaired *t*-tests. Vitamin D serum levels (basal values *vs.* values after one and two months of supplementation, respectively) were compared by paired *t*-tests. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Prism, La Jolla, CA, USA) and SPSS 21.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Quality control of fresh mushrooms

A total of 346 pesticide multiresidues were analysed and all of them were below the detection limit of 0.010 mg kg^{-1}, except fipronil, fipronil sulfone and fipronil sum (<0.0040), ethoprophos (<0.0080), and endrin, aldrin, dieldrin, and dieldrin sum (aldrin plus dieldrin) (<0.0030). Heavy metal contents are shown in Table S1.

3.2. Drying temperature

The two different drying temperatures (40 and 80 °C) used in shiitake resulted in different times to reach a constant mass (72 and 12 h, respectively), but similar dry mass (DM) to fresh mass (FM) ratios were obtained (11.55 ± 0.84 *vs.* 11.87 ± 0.92, respectively). The appearance of the samples was substantially different, because drying at 80 °C led to a darker brown colour in both entire basidiocarps and the resulting powder (Fig. S2). In addition, vitamin D_2 content was significantly
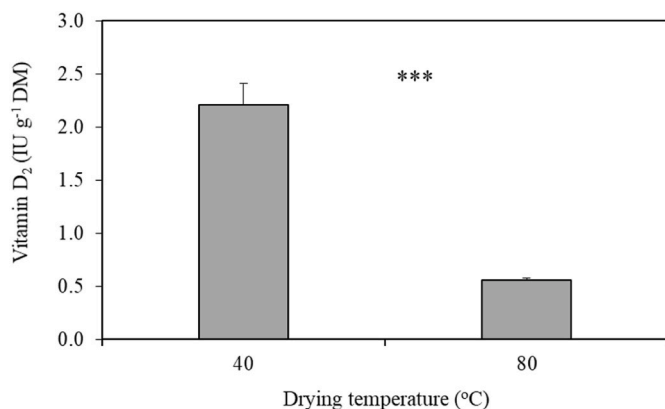
higher (around 4-fold) at 40 than at 80 °C (2.2 ± 0.2 *vs.* 0.52 ± 0.02 IU g^{-1} DM, respectively) (Fig. 1).

3.3. UV-B exposure of shiitake

The global effects of both the UV-B dose and type of treatment (FI, DI, and FDI) on the vitamin D_2 content of the shiitake commercial part were significant ($p < 0.001$). Vitamin D_2 content significantly increased with increasing UV-B dose, but only up to 24 kJ m^{-2} (Fig. 2). Higher contents were obtained under the FI and FDI treatments than under the DI treatment, and the significantly highest content was found under the FDI treatment using a UV-B dose of 24 kJ m^{-2} (Fig. 2). Under these conditions, vitamin D_2 content increased almost 500-fold, from 2.2 IU g^{-1} DM in non-irradiated powder up to around 1000 IU g^{-1} DM in UV-B irradiated powder.

The microbial load of shiitake powder (obtained from the commercial part of the mushroom) was undetectable under most conditions of drying temperature and UV-B dose (Table S2). Only at the lower drying temperature (40 °C) and the lower UV-B doses (9 and 12 kJ m^{-2}), *E. coli*, total coliforms and Enterobacteriaceae were found at low levels, similar to those shown by control samples. In addition, aerobic mesophiles were often found except at the highest UV-B dose applied, showing decreasing counts with increasing doses at both drying temperatures.

The vitamin D_2 content in the shiitake by-products increased with increasing UV-B up to 24 kJ m^{-2}, showing a similar behaviour to the commercial part (Fig. 3). Nevertheless, the vitamin D_2 content in the shiitake by-products was, for each UV-B dose applied, only half of that found in the commercial part (around 600 IU g^{-1} DM), although again almost 500-fold higher than that found in non-UV-B exposed samples.

The microbiological analysis of the powder obtained from shiitake by-products showed the absence of any microbiological problem (Table S3), as occurred with the powder obtained from the commercial part.

3.4. UV-B exposure of king oyster and Portobello button mushrooms

The vitamin D_2 content of king oyster and Portobello button mushrooms samples significantly increased with increasing UV-B dose (Fig. 4). The highest contents were found using the highest dose (24 kJ m^{-2}), and Portobello mushroom showed slightly higher contents than king oyster and shiitake. The microbiological quality was satisfactory in the UV-B irradiated samples in comparison with the non-irradiated samples (Table S4).
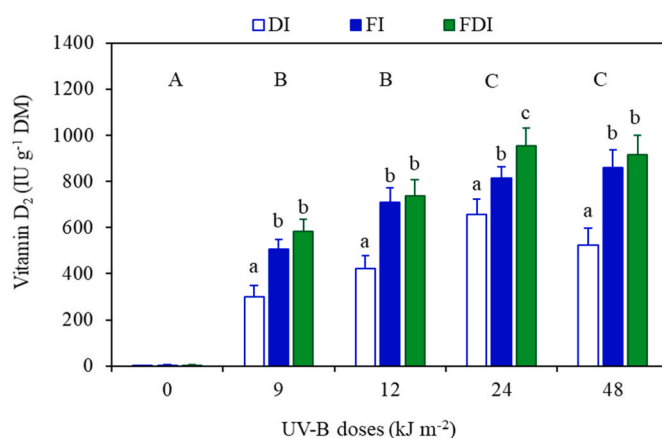

Fig. 1. Effect of the drying temperature on the vitamin D_2 content in shiitake slices. Means ± SE are shown (n = 3), together with the significance of the Student's *t*-test between the two temperatures (***, $p < 0.001$).

Fig. 2. Effect of the applied UV-B dose on the vitamin D_2 content of the shiitake commercial part for each treatment (DI, dry irradiation; FI, fresh irradiation; FDI, fresh + dry irradiation). Means ± SE are shown (n = 3). Different capital and lower case letters mean, respectively, significant differences between UV-B doses and significant differences between treatments for each dose (Tukey's test after a two-way ANOVA).
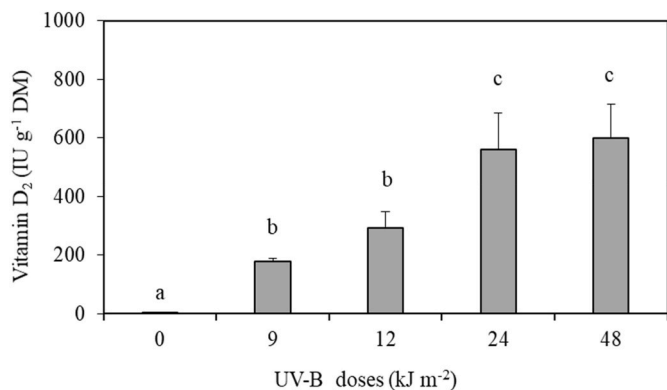
Fig. 3. Effect of the applied UV-B dose on the vitamin D_2 content of the shiitake by-products (stalk bases and not commercially useable individuals) for the FDI treatment (fresh + dry irradiation). Means ± SE are shown (n = 3). Different letters mean significant differences between UV-B doses (Tukey's test after a one-way ANOVA).
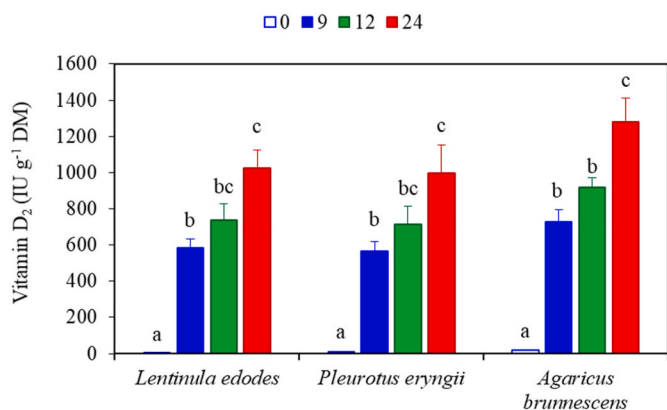

Fig. 4. Effect of the applied UV-B dose (0–24 kJ m^{-2}), following the FDI treatment (fresh + dry irradiation), on the vitamin D_2 content of the commercial parts of the three mushroom species studied. Means ± SE are shown (n = 3). Different letters mean significant differences between UV-B doses for each species (Tukey's test after a one-way ANOVA for each species). ns: not significant.

3.5. Characteristics of encapsulated shiitake powder for the bioavailability study

The shiitake material encapsulated for the bioavailability study had been exposed to the treatment previously found to be the most efficient to increase the vitamin D_2 content (FDI treatment using a UV-B dose of 24 kJ m^{-2}). Specifically, the encapsulated powder had a vitamin D_2 content of around 1092 ± 102 IU g^{-1} DM. Given that each capsule contained around 600 mg of powder, it provided around 600 IU of vitamin D_2, within the range recommended for the general population (600–800 IU per day). The vitamin D_2 content in the encapsulated shiitake powder did not change after storage for 6 months at room temperature (Fig. 5). The microbial load of the powder at the beginning of the bioavailability study was low, both in the placebo and the UV-B irradiated powder, and mainly made of aerobic mesophiles (Table S5). The nutritional composition of the shiitake powder was similar between the placebo and the UV-B irradiated samples, except vitamin D_2, which increased 500-fold in the irradiated powder (Table S6).

3.6. Vitamin D_2 bioavailability in humans after consumption of enriched mushroom capsules

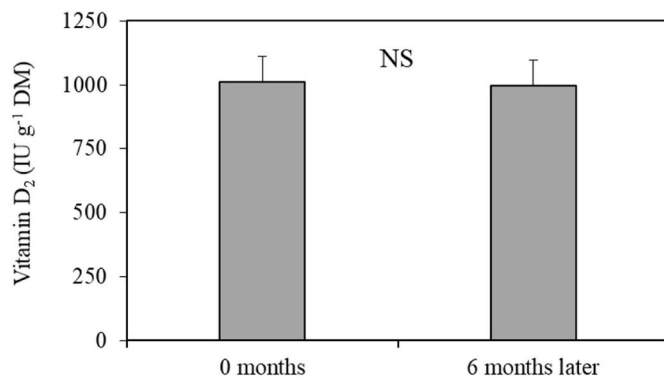The placebo and vitamin-D groups were well-matched in terms of

age, gender and body mass index (Table S7). Mean 25-hydroxyvitamin D serum levels were within the normal range (>20 ng mL^{-1} following NIH, 2023) at the beginning of the study (Fig. 6A), but significantly decreased

Fig. 5. Vitamin D_2 content in the encapsulated shiitake powder at the moment of encapsulation and after storage for 6 months at room temperature. Means ± SE are shown (n = 3), together with the significance of the Student's t-test between the two moments (NS, not significant).
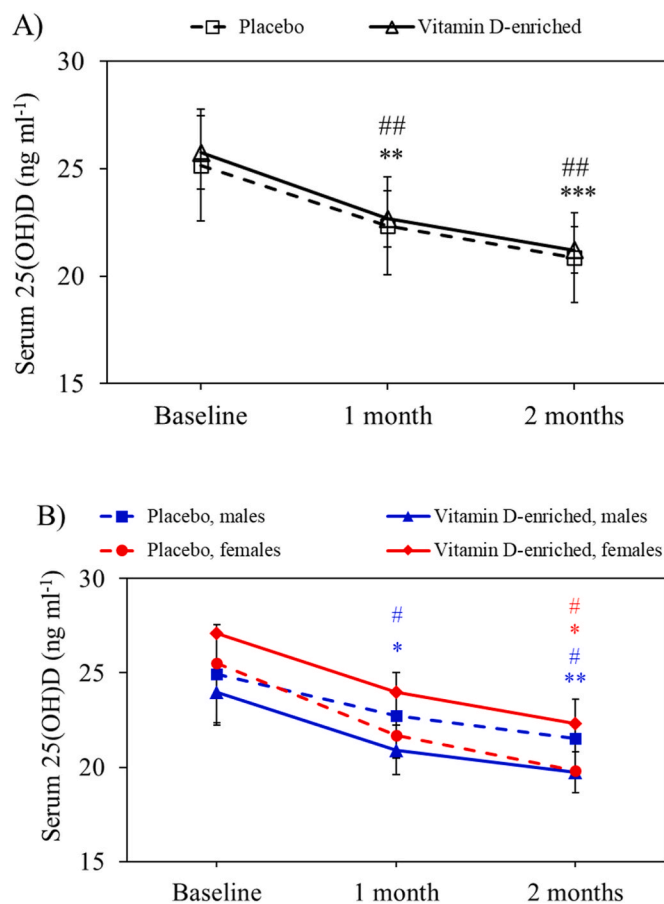

Fig. 6. Serum 25-hydroxyvitamin D levels in the two groups of participants in the bioavailability study (placebo and vitamin D-enriched groups) in three moments of the study (at the beginning (baseline) and after one and two months of supplement administration), either considering A) all participants together, and B) participants differentiated by gender (blue, males; red, females). Means ± SE are shown. Significant differences between baseline and the other two moments are shown for the placebo group (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) and for the vitamin-D enriched group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

during the winter months as compared to baseline values (12% decrease in January and 18% decrease in February), when all participants were analysed as a whole. When participants were split into placebo or vitamin-D groups (Fig. 6A), mean levels did not significantly differ among the groups at the beginning of the study. However, a significant decrease was again observed after one and/or two months of supplementation compared to the basal values. The decrease was stronger in the placebo group ($p < 0.001$ *vs.* corresponding basal values at the end of the experimental period). The decrease was similar in both males and females, with no statistically significant differences between both genders. However, such decrease was statistically significant since the first month in males but only at the end of the experimental period in females, independently of having been supplemented with vitamin D_2-enriched mushroom capsules or not (Fig. 6B).

Serum 25-hydroxyvitamin D levels were also analysed taking into account the baseline status of each individual (Fig. 7), as in Cashman, Kiely, Seamans, and Urbain (2016). Thus, participants were divided according to their median value of serum vitamin D levels (23.2 ng mL^{-1}) (Fig. 7A) or to the current baseline levels clinically accepted (20 ng mL^{-1}, following NIH, 2023) (Fig. 7B). After two months of treatment, the placebo and the vitamin-D groups showed similar decreases when the serum vitamin levels were higher than the median or the accepted clinical level. However, when the serum vitamin levels were lower than those limits, the decrease was significantly lower in the vitamin-D group than in the placebo.

To test if the results observed had been affected by the degree of compliance, the total number of capsules ingested by each participant was registered. Three subjects did not ingest the total number of capsules due to omission and/or gastrointestinal discomfort (96.5% adherence) in the placebo group. Similarly, four people did not take the total number of capsules required (99.4% adherence) in the vitamin D_2-enriched group. Thus, no statistically significant differences were observed among both groups.

Some adverse reactions were reported by the participants after two months of nutritional supplementation (Table S8). The most frequent symptoms were gastrointestinal discomfort, being slightly more frequent in the placebo than in the vitamin-D group ($p = 0.06$), suggesting that these effects could be due to the mushroom intake *per se* and not because of vitamin D_2 enrichment. Finally, the amount of vitamin D ingested through food and/or dietary supplements (different from the capsules used in our study) did not differ between the placebo and the vitamin-D groups (Fig. S8).

4. Discussion

The fresh mushrooms used in the present study showed a high quality in terms of pesticide and heavy metal contents, which were below legal thresholds (European Union, 2021a; 2021b). This fact, together with the low microbial load found in the vitamin D_2-enriched shiitake powder, made it safe and suitable to be tested as a food supplement in humans. Contrary to UV-C radiation, there are few data on the effects of UV-B on the microbial load of mushrooms. Kalaras, Beelman, and Elias (2012) did not find any effect in *Agaricus bisporus*, which was attributed to the low UV-B level applied in their experiments. In our study, even considering the very low microbial load and the practical absence of potentially pathogenic microbes in the raw mushrooms, the microbial load decreased as UV-B increased, but we applied higher levels than Kalaras, Beelman, and Elias (2012). Under the conditions applied in our study, we only found a reduced presence of aerobic mesophiles, which represent different non-pathogenic bacteria with no microbiological risk. In addition, all the samples analysed showed much lower counts than those allowed by the Spanish legislation (10^7 cfu g^{-1} in food prepared from raw vegetables: BOE, 2000).

Following previous studies (Phillips et al., 2011), the mean vitamin D_2 content in fresh shiitake is 17 IU per 100 g. On this basis, the expected content after drying, considering a water loss of around 90%, would be
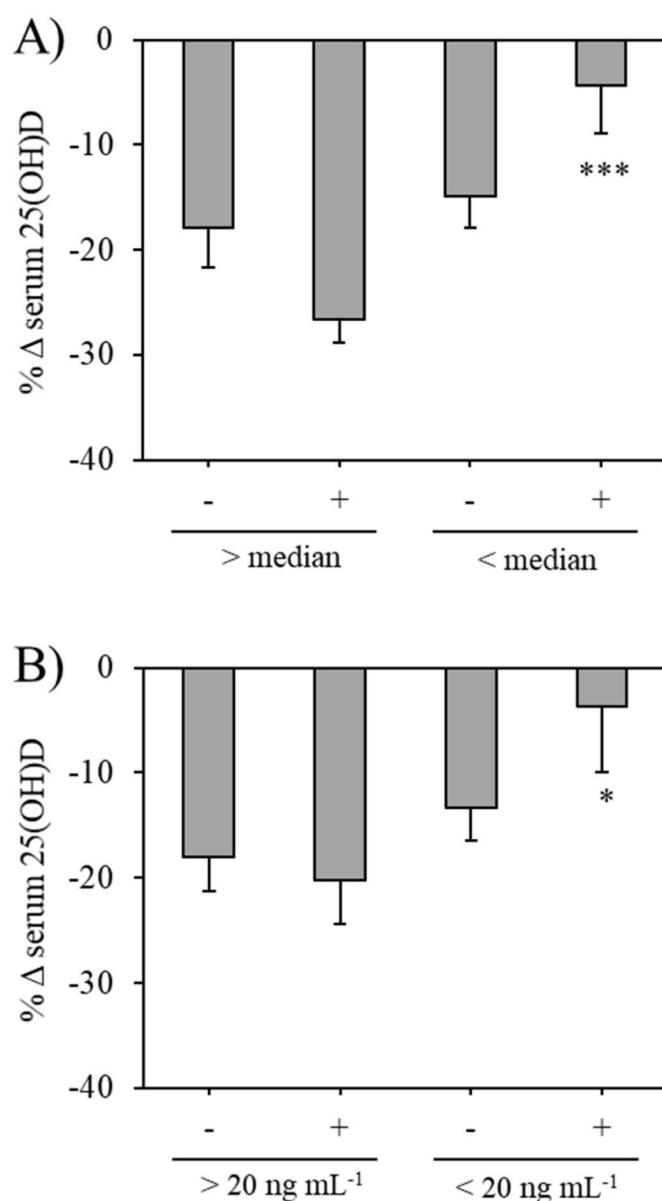

Fig. 7. Changes in serum 25-hydroxyvitamin D (ng mL^{-1}) levels in the two groups of participants in the bioavailability study (placebo group ($-$) and vitamin D-enriched group ($+$)) after two months of supplement administration, depending on their baseline serum vitamin D levels expressed as A) the median of the total serum levels measured (23.2 ng mL^{-1}) or B) the normality cut-off value accepted in clinical guidelines (20 ng mL^{-1}). Significant differences between the placebo and vitamin D-enriched groups are shown (*$p < 0.05$, ***$p < 0.001$). Means ± SE are shown.

approximately 2.00 IU g^{-1} DM. In our case, this content was reached or exceeded only after drying at 40 °C (2.20 ± 0.20 IU g^{-1} DM), whereas drying at 80 °C led to both a lower vitamin D_2 content (0.52 ± 0.02 IU g^{-1} DM) and worse sensory characteristics of the mushrooms. Other authors used similar drying temperatures and reported the importance of drying at moderate temperatures to retain higher vitamin D_2 levels (Gallotti & Lavelli, 2020). Mushroom dehydration by freeze-drying has been proposed to better preserve vitamin D_2 levels (Lee & Aan, 2016; Urbain et al., 2016), but this technology may not be usually available for mushroom producers.

UV-B irradiation of shiitake resulted in an increase in vitamin D_2 content. The content achieved (580 IU g^{-1} DM) by using the lowest UV-B dose (9 kJ m^{-2}) was much higher than the contents typical of food of

animal origin particularly rich in vitamin D_2, such as salmon (5 IU g^{-1}), egg yolk (3.5), or even cod liver oil (87) (BEDCA, 2023). In addition, the vitamin D_2 increase in shiitake was directly related to the UV-B dose applied up to 24 kJ m^{-2}. Thus, it would be possible to control the desired final content, avoiding of a potential overdose which could cause hypercalcemia (Marriott, 1997).

The highest increase in vitamin D_2 content in shiitake was found when UV-B was applied in two different moments of the mushroom processing (75% of the total dose on the fresh product and the remaining 25% on the dry product). Different authors have reported increases in vitamin D_2 content by irradiating mushroom powder or dehydrated mushrooms (Heo et al., 2020; Lee & Aan, 2016; Morales et al., 2017). All these results may contradict the finding of Jasinghe and Perera (2005) that the product should be subjected to UV irradiation before its moisture content drops below 70% on a fresh mass basis.

Our selected protocol (drying at 40 °C and application of a UV-B dose of 24 kJ m^{-2} distributed in two different processing stages) resulted in a vitamin D_2 content around 1000 IU g^{-1} DM in shiitake, but different protocols may lead to different results, also depending on the species used (Kamweru & Tindibale, 2016). Ko et al. (2008) irradiated fresh shiitake and white button mushrooms, obtaining an increase in vitamin D_2 proportional to the UV-B dose applied. However, a higher dose than ours (50 kJ m^{-2}) was required to obtain vitamin D_2 contents higher than 1000 IU g^{-1} DM. In white button mushrooms, Mau, Chen, and Yang (1998) showed that a 2-h UV-B exposure (using 0.14 mW cm^{-2} irradiance, for a total UV-B dose of 10 kJ m^{-2}) resulted in a vitamin D_2 content of 500 IU g^{-1} DM. In white button and oyster mushrooms, Gallotti and Lavelli (2020) reported very high vitamin D_2 contents (2200 IU g^{-1} DM) by applying 0.4 W m^{-2} UV-B irradiance, but again the irradiation period was longer than ours (24 h). These studies used relatively low UV-B irradiances, and the associated longer exposure times are impractical for commercial mushroom production. In the opposite extreme, Roberts et al. (2008) reduced the exposure period of Portobello mushrooms to only 8 min (using an irradiance of 1.0 mW cm^{-2} and a dose of 0.5 J cm^{-2}), but they only obtained a vitamin D_2 content of 150 IU g^{-1} DM. Overall, our protocol for shiitake achieved a more adequate balance between UV-B irradiance (16 W m^{-2}), exposure time (25 min) and final vitamin D_2 content (1000 IU g^{-1} DM), being thus more efficient for commercial production. In addition, the same protocol was applied to other species (king oyster and Portobello mushrooms) with similar successful results.

The results obtained in the shiitake by-products may be of special practical interest. The vitamin D_2 increase in UV-B treated by-products was similar to that obtained in the commercial part of the mushrooms (around 500-fold the content in untreated samples). However, the final vitamin D_2 content (about 600 IU g^{-1} DM) was lower, probably due to the uneven distribution of the vitamin precursors in the different parts of the basidiocarp. The highest content of vitamin D_2 is found in the gills and, consequently, the content in the commercial part (mainly the pileus or cap) can be 4-fold higher than that in the stalks (Jasinghe & Perera, 2005; Ko et al., 2008). Despite this fact, the producers could earn an additional profit from the sale of the vitamin-enriched shiitake by-products as a complement for animal feed. In particular, vitamin D_2 supplements are especially adequate for stabled cattle which are not directly exposed to natural solar radiation (MarketsandMarkets, 2023). In addition, the producers would prevent the cost of residue removal and processing.

Mushrooms exposed to UV radiation increase their vitamin D_2 content, but can also produce nonvitamin products, such as lumisterol and tachysterol, whose effects on human health are insufficiently known (Kalaras, Beelman, Holick, & Elias, 2012; Schümmer, Stangl, & Wätjen, 2021; Wittig, Krings, & Berger, 2013). Given the experimental conditions used, the presence of these photoproducts in the UV-B-irradiated mushrooms produced in our study is very unlikely. In addition, we did not find them in our analyses (Fig. S5). Nevertheless, the occurrence of these potentially dangerous compounds in UV-irradiated mushrooms, as

well as their biological activities, should be considered in future studies.

Overall, the designed facility for the UV-B irradiation of mushrooms met the proposed objective of being efficient and versatile to test different irradiances and exposure times in diverse mushroom species, and it was also suitable to irradiate mushroom by-products. In addition, although UV-B is not the most aggressive form of radiation, the facility would comply with all the safety measures for the operators (Salemi et al., 2021).

Additionally to its microbiological quality, it was verified that the nutritional characteristics of the shiitake powder used for encapsulation was not modified by the UV-B exposure, except for the increase in vitamin D_2. This agreed with the results reported by Simon, Phillips, Horst, and Munro (2011). Furthermore, we confirmed that the vitamin D_2 content in the capsules was stable along time (six-month storage period), which coincided with previous results by Roberts et al. (2008) and Salemi et al. (2021). Specifically, the last authors reported a low degradation rate constant (0.025 h^{-1}). This is of great interest for the potential distribution and commercialization of the capsules, also considering that a single capsule of the easy-to-ingest food supplement prepared in our study would provide the recommended minimum daily intake for an adult (NIH, 2023).

Regarding the bioavailability study, the ingestion of the vitamin D_2-enriched shiitake powder was ineffective in improving serum 25-hydroxyvitamin D status in humans. These results contrasted with previous studies (Biancuzzo, Clarke, Reitz, Travison, & Holick, 2013; Keegan, Lu, Bogusz, Williams, & Holick, 2013; Urban, Singler, Ihorst, Biesalski, & Bertz, 2011). These discrepancies could be explained by (1) the limited number of volunteers included in each group; (2) the lower doses used in our study compared to others (600 IU per capsule *vs.* 2000–4000 IU); and (3) we herein measured total 25-hydroxyvitamin D vitamin, while several studies have observed that vitamin D_2 from enriched mushrooms increased serum 25-hydroxyvitamin D_2 concentrations with no significant effect on 25-hydroxyvitamin D_3 or total 25-hydroxyvitamin D. Therefore, it is plausible that vitamin D_2 supplementation did not affect vitamin D status because 25-hydroxyvitamin D_2 concentrations increased but 25-hydroxyvitamin D_3 decreased proportionally (Nieman et al., 2014; Stepien et al., 2013). The impact of other factors such as cooking should also be taken into account to increase the bioavailability of vitamin D from UV-B-irradiated mushrooms (Cardwell et al., 2018).

Interestingly, the decrease observed on total serum 25-hydroxyvitamin D due to winter conditions was less strong when the mean baseline vitamin D status of the participants was inadequate (less than 20 ng mL^{-1} or lower than the median). This suggests that the vitamin D-enriched capsules could exert some "protective" effects, similarly to what has already been demonstrated by others (Cashman et al., 2016). Further research is needed to explore whether this potential protective action has any physiological importance.

5. Conclusion

We have developed a technology, easy to export to the market, which allows to produce different mushrooms (shiitake, king oyster and Portobello button) enriched in vitamin D_2 through the use of UV-B radiation under controlled conditions. In shiitake, UV-B treatment induced a significant increase in the vitamin D_2 content not only in the commercial part of the mushroom, but also in the by-products. This material is usually discarded in the production process but could be valorized for animal feed. Our method allowed to obtain a potentially healthy nutritional supplement (due to its high vitamin D_2 content) in an economical and sustainable way, in line with the concept of bioeconomy of the European Union. The bioavailability results obtained open the door to future studies with higher UV-B doses and/or new ways of cooking mushrooms fortified with vitamin D_2. In addition, the vitamin D_2 supplements produced could be administered to stratified people, on the basis of their baseline vitamin D concentrations or their specific

pathologies associated with low plasma levels of this vitamin, to better fight against the alarming deficiency of vitamin D in humans.

CRediT authorship contribution statement

Raquel Hidalgo-Sanz: Investigation, Formal analysis, Writing – original draft. **María-Ángeles Del-Castillo-Alonso:** Investigation, Formal analysis, Visualization, Writing – original draft. **Laura Monforte:** Investigation. **Rafael Tomás-Las-Heras:** Investigation. **Susana Sanz:** Formal analysis, Writing – original draft. **Carmen Olarte:** Formal analysis, Investigation. **Patricia Pérez-Matute:** Investigation, Formal analysis, Visualization, Writing – original draft. **María Íniguez-Martí-nez:** Investigation, Visualization, Writing – original draft. **Alexandrina-Laura Ene:** Investigation. **Javier Martínez-Abaigar:** Conceptualiza-tion, Funding acquisition, Writing – review & editing. **Encarnación Núñez-Olivera:** Conceptualization, Investigation, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115210.

References

AOAC. (1990). *Official methods of analysis* (15th ed.). Washington DC: Association of Official Analytical Chemists.

Atlas Big. (2023). Retrieved from https://www.atlasbig.com/en-us/countries-mushroom -truffle-production Accessed May 15, 2023.

Barnes, P. W., Robson, T. M., Zepp, R. G., Bornman, J. F., Jansen, M. A. K., Ossola, R., et al. (2023). Interactive effects of changes in UV radiation and climate on terrestrial ecosystems, biogeochemical cycles, and feedbacks to the climate system. *Photochemical and Photobiological Sciences*. https://doi.org/10.1007/s43630-023-00376-7

BEDCA. (2023). *Base de Datos Española de Composición de Alimentos*. Retrieved from https://www.bedca.net/. (Accessed 27 April 2023).

Biancuzzo, R. M., Clarke, N., Reitz, R. E., Travison, T. G., & Holick, M. F. (2013). Serum concentrations of 1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3 in response to vitamin D_2 and vitamin D_3 supplementation. *Journal of Clinical Endocrinology and Metabolism, 98*, 973–979. https://doi.org/10.1210/jc.2012-2114

BOE (Boletín Oficial del Estado). (2000). *Real Decreto 3484/2000, de 29 de diciembre, por el que se establecen las normas de higiene para la elaboración, distribución y comercio de comidas preparadas*.

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. https://doi.org/10.3390/nu10101498

Cashman, K. D., Kiely, M., Seamans, K. M., & Urbain, P. (2016). Effect of ultraviolet light-exposed mushrooms on vitamin D status: Liquid chromatography-tandem mass spectrometry reanalysis of biobanked sera from a randomized controlled trial and a systematic review plus meta-analysis. *The Journal of Nutrition, 146*, 565–575. https://doi.org/10.3945/jn.115.223784

CPAER. (2023). *Consejo de la Producción Agraria Ecológica de La Rioja*. Retrieved from https://www.cpaer.org/es/public/certificate#punto-legislacion. (Accessed 17 July 2023).

European Union. (2018). *Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation*. EC) No 834/2007.

European Union. (2021a). *Commission Regulation (EU) 2021/1317 of 9 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs*.

European Union. (2021b). *Commission Regulation (EU) 2021/1323 of 10 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in certain foodstuffs*.

Gallotti, F., & Lavelli, V. (2020). The effect of UV irradiation on vitamin D_2 content and antioxidant and antiglycation activities of mushrooms. *Foods, 9*, 1087. https://doi.org/10.3390/foods9081087

Grant, W. B., Lahore, H., McDonnell, S. L., Baggerly, C. A., French, C. B., Aliano, J. L., et al. (2020). Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients, 12*, 988. https://doi.org/10.3390/nu12040988

Heo, T. Y., Kim, Y. N., Park, I. B., & Lee, D. U. (2020). Amplification of vitamin D_2 in the white button mushroom (*Agaricus bisporus*) by UV-B irradiation and jet-milling for its potential use as a functional ingredient. *Foods, 9*, 1713. https://doi.org/10.3390/foods9111713

Holick, M. F. (2017). The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Reviews in Endocrine & Metabolic Disorders, 18*, 153–165. https://doi.org/10.1007/s11154-017-9424-1

Hu, D., Chen, W., Li, X., Yue, T., Zhang, Z., Feng, Z., et al. (2020). Ultraviolet irradiation increased the concentration of vitamin D_2 and decreased the concentration of ergosterol in shiitake mushroom (*Lentinus edodes*) and oyster mushroom (*Pleurotus ostreatus*) powder in ethanol suspension. *ACS Omega, 5*, 7361–7368. https://doi.org/10.1021/acsomega.9b04321

ISO. (2019). Retrieved from https://www.iso.org/home.html. Accessed November 10, 2019.

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. https://doi.org/10.1016/j.foodchem.2004.08.022

Kalaras, M. D., Beelman, R. B., & Elias, R. J. (2012). Effects of postharvest pulsed UV light treatment of white button mushrooms (*Agaricus bisporus*) on vitamin D_2 content and quality attributes. *Journal of Agricultural and Food Chemistry, 60*, 220–225. https://doi.org/10.1021/jf203825e

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. https://doi.org/10.1016/j.foodchem.2012.04.132

Kamweru, P. K., & Tindibale, E. L. (2016). Vitamin D and vitamin D from ultraviolet-irradiated mushrooms. *International Journal of Medicinal Mushrooms, 18*, 205–214. https://doi.org/10.1615/IntJMedMushrooms.v18.i3.30

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. https://doi.org/10.4161/derm.23321

Ko, J. A., Lee, B. H., Lee, J. S., & Park, H. J. (2008). Effect of UV-B exposure on the concentration of vitamin D_2 in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry, 56*, 3671–3674. https://doi.org/10.1021/jf073398s

Kudam. (2023). Retrieved from https://www.kudam.com/wp-content/uploads/sites/91/2022/12/R-356.MENU-DE-SERVICIOS-Rev-13.pdf. Accessed July 17, 2023.

Lee, N. K., & Aan, B. Y. (2016). Optimization of ergosterol to vitamin D_2 synthesis in *Agaricus bisporus* powder using ultraviolet-B radiation. *Food Science and Biotechnology, 25*, 1627–1631. https://doi.org/10.1007/s10068-016-0250-0

MarketsandMarkets. (2023). *Global vitamin D market by analog (vitamin D2, vitamin D3), form (dry, liquid), application (feed & pet food, pharma, functional food, and personal care), end users (adults, pregnant women, and children), IU Strength and Region - forecast to 2027*. Retrieved from https://www.marketresearch.com/MarketsandMarkets-v3719/Vitamin-Analog-D2-D3-Form-32767313/. (Accessed 6 March 2023).

Marriott, B. M. (1997). Vitamin D supplementation: A word of caution. *Annals of Internal Medicine, 127*, 231–233. https://doi.org/10.7326/0003-4819-127-3-199708010-00009

Mau, J. L., Chen, P. R., & Yang, J. H. (1998). Ultraviolet irradiation increased vitamin D_2 content in edible mushrooms. *Journal of Agricultural and Food Chemistry, 46*, 5269–5272. https://doi.org/10.1021/jf980602q

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 & Emerging Technologies, 41*, 330–336. https://doi.org/10.1016/j.ifset.2017.04.008

Neale, R. E., Lucas, R. M., Byrne, S. N., Hollestein, L., Rhodes, L. E., Yazar, S., et al. (2023). The effects of exposure to solar radiation on human health. *Photochemical and Photobiological Sciences*. https://doi.org/10.1007/s43630-023-00375-8

Neill, H. R., Gill, C. I. R., McDonald, E. J., McRoberts, W. C., & Pourshahidi, L. K. (2023). The future is bright: Biofortification of common foods can improve vitamin D status. *Critical Reviews in Food Science and Nutrition, 63*, 505–521. https://doi.org/10.1080/10408398.2021.1950609

Nieman, D. C., Gillitt, N. D., Shanely, R. A., Dew, D., Meaney, M. P., & Luo, B. (2014). Vitamin D_2 supplementation amplifies eccentric exercise-induced muscle damage in NASCAR pit crew athletes. *Nutrients, 6*, 63–75. https://doi.org/10.3390/nu6010063

NIH. (2023). *National Institutes of Health*. Retrieved from https://ods.od.nih.gov/fact sheets/VitaminD-HealthProfessional. (Accessed 15 May 2023).

Phillips, K. M., Ruggio, D. M., Horst, R. L., Minor, B., Simon, R. R., Feeney, M. J., et al. (2011). Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. *Journal of Agricultural and Food Chemistry, 59*, 7841–7853. https://doi.org/10.1021/jf104246z

Roberts, J. S., Teichert, A., & McHugh, T. H. (2008). Vitamin D_2 formation from post-harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. *Journal of Agricultural and Food Chemistry, 56*, 4541–4544. https://doi.org/10.1021/jf0732511

Robson, T. M., Aphalo, P. J., Banas, A. K., Barnes, P. W., Brelsford, C. C., Jenkins, G. I., et al. (2019). A perspective on ecologically relevant plant-UV research and its practical application. *Photochemical and Photobiological Sciences, 18*, 970–988. https://doi.org/10.1039/c8pp00526e

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., et al. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: What clinicians need to know. *Journal of Clinical Endocrinology & Metabolism, 96*, 53–58. https://doi.org/10.1210/jc.2010-2704

Salemi, S., Saedisomeolia, A., Azimi, F., Zolfigol, S., Mohajerani, E., Mohammadi, M., et al. (2021). Optimizing the production of vitamin D in white button mushrooms (*Agaricus bisporus*) using ultraviolet radiation and measurement of its stability. *Lebensmittel-Wissenschaft und -Technologie, 137*, Article 110401. https://doi.org/10.1016/j.lwt.2020.110401

Sapozhnikova, Y., Byrdwell, W. C., Lobato, A., & Romig, B. (2014). Effects of UV-B radiation levels on concentrations of phytosterols, ergothioneine, and polyphenolic compounds in mushroom powders used as dietary supplements. *Journal of Agricultural and Food Chemistry, 62*, 3034–3042. https://doi.org/10.1021/jf403852k

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. https://doi.org/10.3390/foods10123142

Simon, R. R., Phillips, K. M., Horst, R. L., & Munro, I. C. (2011). Vitamin D mushrooms: Comparison of the composition of button mushrooms (*Agaricus bisporus*) treated postharvest with UVB light or sunlight. *Journal of Agricultural and Food Chemistry, 59*, 8724–8732. https://doi.org/10.1021/jf201255b

Singh, U., Gautam, A., & Sharma, S. (2021). Enhancement of Vitamin D_2 content through ultraviolet-B irradiation in submerged cultivated *Pleurotus eryngii* mycelia using response surface methodology. *Journal of Applied Biology & Biotechnology, 9*, 121–126. https://doi.org/10.7324/JABB.2021.9315

Stepien, M., O'Mahony, L., O'Sullivan, A., Collier, J., Fraser, W. D., Gibney, M. J., et al. (2013). Effect of supplementation with vitamin D_2-enhanced mushrooms on vitamin D status in healthy adults. *Journal of Nutrition Sciences, 2*, e29. https://doi.org/10.1017/jns.2013.22

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. https://doi.org/10.1021/acs.jafc.5b02945

Urbain, P., Singler, F., Ihorst, G., Biesalski, H. K., & Bertz, H. (2011). Bioavailability of vitamin D_2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: A randomized controlled trial. *European Journal of Clinical Nutrition, 65*, 965–971. https://doi.org/10.1038/ejcn.2011.53

Urbain, P., Valverde, J., & Jakobsen, J. (2016). Impact on vitamin D_2, vitamin D_4 and agaritine in *Agaricus bisporus* mushrooms after artificial and natural solar UV light exposure. *Plant Foods for Human Nutrition, 71*, 314–321. https://doi.org/10.1007/s11130-016-0562-5

Villares, A., Mateo-Vivaracho, L., García-Lafuente, A., & Guillamón, E. (2014). Storage temperature and UV-irradiation influence on the ergosterol content in edible mushrooms. *Food Chemistry, 147*, 252–256. https://doi.org/10.1016/j.foodchem.2013.09.144

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. https://doi.org/10.1016/j.jfca.2013.05.017