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# UV-B biofortification of edible mushrooms: mechanistic insights, bioavailability, and potential to mitigate vitamin d deficiency

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**Abstract**
Vitamin D deficiency is a serious global public health concern, caused by inadequate solar exposure, urban lifestyles, insufficient dietary intake, and inconsistent fortification procedures. Vitamin D from natural foods is still scarce, especially among cultures that eat mostly plant-based diets. Edible mushrooms contain ergosterol, a photochemical precursor of vitamin $D_2$ (ergocalciferol), making them a unique source of nourishment. When exposed to UV-B radiation (280-315 nm), ergosterol opens its photolytic ring to create pre-vitamin $D_2$, which is then thermally isomerised to vitamin $D_2$. This review critically synthesises current findings on UV-B biofortification of mushrooms, taking into account mechanistic photochemistry, processing, analytical, and translational issues. Key determinants of vitamin $D_2$ yield including species variability, tissue morphology, moisture content, UV dose and irradiance, exposure geometry, and post-irradiation equilibration are evaluated to explain heterogeneity across studies and to identify process optimisation constraints. This study examines the post-harvest stability of vitamin $D_2$ throughout refrigerated storage, drying, and cooking. Packaging, light exposure, and processing conditions are crucial for nutrient retention. *In vitro* digestive models and human intervention studies show that mushroom-derived vitamin $D_2$ is bioavailable and can boost circulation 25-hydroxyvitamin D levels, but its efficacy compared to vitamin $D_3$ is still contested. Finally, we investigate industrial implementation paths, regulatory considerations, and the incorporation of vitamin $D_2$-enriched mushroom components into food fortification programs. Overall, UV-B-biofortified mushrooms are a mechanistically sound and scalable technique for increasing dietary vitamin D, but they require further standardisation and clinical validation to have a population-wide impact.

**Keywords:** UV-B irradiation, mushroom biofortification, ergosterol photoconversion, vitamin $D_2$ (ergocalciferol), bioavailability

## Introduction
Vitamin D deficiency has emerged as one of the most prevalent micronutrient insufficiencies worldwide, transcending geographic, socioeconomic, and demographic boundaries. Traditionally viewed as a nutrient produced endogenously through cutaneous photoconversion of 7-dehydrocholesterol under ultraviolet-B (UV-B) radiation, vitamin D is now increasingly recognised within a wider nutritional and food systems context. Modern lifestyle patterns including urbanisation, indoor occupational environments, widespread sunscreen use, air pollution, and cultural clothing practices have significantly reduced effective sunlight exposure in many populations (Cui et al., 2023) [14]. Consequently, dietary sources have gained greater importance in maintaining adequate vitamin D status, yet natural food sources remain scarce. Fatty fish, fortified dairy products, and egg yolk are the primary sources in most diets, however these foods are not universally accessible, culturally acceptable, or environmentally sustainable (Alam et al., 2026) [2]. Within this nutritional landscape, edible mushrooms have attracted significant scientific attention as a unique, non-animal dietary source capable of providing substantial amounts of vitamin $D_2$ when exposed to UV-B radiation.
The interest in mushrooms as vehicles for vitamin D biofortification arises from a unique biochemical characteristic: the presence of ergosterol within fungal cell membranes.

Ergosterol functions as the fungal analogue of cholesterol in animals and serves as the photochemical precursor of vitamin $D_2$ (ergocalciferol). When exposed to UV-B radiation within wavelengths typically between 280 and 315 nm, ergosterol undergoes photolysis to form pre-vitamin $D_2$,which subsequently undergoes thermally induced isomerisation to vitamin $D_2$ (Sommer *et al.*, 2023) [53]. This photochemical transformation parallels the cutaneous synthesis of vitamin $D_3$ in humans and represents one of the few naturally occurring pathways through which a plantderived food can generate nutritionally meaningful quantities of vitamin D. Importantly, the conversion process can occur both during mushroom growth under sunlight exposure and through post-harvest UV treatment, providing an opportunity to intentionally enhance vitamin $D_2$ concentrations through controlled technological interventions.

Over the past two decades, advances in food photobiology and post-harvest technology have enabled systematic exploration of UV-B irradiation as a biofortification strategy. Controlled exposure of harvested mushrooms to UV-B light can increase vitamin $D_2$ concentrations by several orders of magnitude relative to untreated samples, often reaching levels comparable to those found in fortified foods (Hidalgo-Sanz *et al.*, 2023) [22]. Such interventions are particularly attractive because they rely on physical processing rather than chemical fortification, aligning with consumer preferences for minimally processed or "natural" functional foods. Moreover, the scalability of UV treatment ranging from small laboratory systems to industrial conveyorbased irradiation units suggests potential for integration into existing mushroom production chains (Sharma *et al.*, 2024) [50].

Despite the apparent simplicity of the ergosterol-vitamin $D_2$ photoconversion pathway, the efficiency of vitamin $D_2$ generation in mushrooms is influenced by a complex interplay of biological, physical, and environmental factors. Species-specific differences in ergosterol content, morphological features affecting light penetration, moisture content, and post-harvest physiology all modulate photochemical conversion efficiency (Saroj and Xiong 2025) [49]. Additionally, parameters such as UV-B wavelength spectrum, irradiation intensity, exposure duration, and orientation of mushroom tissues contribute significantly to the final vitamin $D_2$ yield. The resulting variability across studies has generated both enthusiasm and uncertainty regarding optimal processing conditions and the reproducibility of biofortification outcomes (Kaur *et al.*, 2023) [28].

The integration of nutritional epidemiology, fungal biochemistry, and food processing studies underscores UV-B-mediated mushroom biofortification as an effective food-based approach to combat vitamin D deficiency. However, the transition from laboratory discoveries to extensive nutritional interventions necessitates a comprehensive understanding of the fundamental photobiochemical principles, dependable testing techniques, and substantiated facts about the stability and bioavailability of vitamin $D_2$.. This review consolidates existing knowledge on the photochemical transformation of ergosterol to vitamin $D_2$ in mushrooms, the factors affecting its production, methods for quantification, and new findings regarding its bioavailability and health significance.

**Global Burden of Vitamin D Deficiency**

Vitamin D deficiency has evolved from a regionally recognised nutritional concern into a global public health challenge affecting populations across diverse climatic zones, socioeconomic strata, and age groups. Epidemiological assessments conducted over the past two decades consistently indicate that suboptimal serum 25-hydroxyvitamin D [25(OH)D] concentrations are widespread, with estimates suggesting that approximately one billion individuals worldwide exhibit biochemical deficiency or insufficiency depending on diagnostic thresholds (Cui *et al.*, 2023) [14]. Large pooled analyses show that approximately 15-20% of the global population has serum 25(OH)D levels below 30 nmol/L, and nearly half fall below 50 nmol/L, the threshold commonly used to define insufficiency (Van Schoor *et al.*, 2024) [60]. However, prevalence estimates vary due to differences in cut-off values and assay methodologies. These numbers highlight the severity of the deficiency and its persistence despite decades of increased public health awareness.

The geographical distribution of deficiency shows a paradoxical pattern: vitamin D insufficiency is prevalent even in regions with abundant sunlight. South Asia, the Middle East, and parts of North Africa report some of the highest prevalence rates, often exceeding 60-70% in specific demographic groups (Machuron *et al.*, 2025) [33]. Multiple interacting factors explain this phenomenon. While traditional clothing practices restrict the amount of skin surface area exposed to sunlight, urbanisation and indoor lifestyles significantly reduce UV exposure (Dubey *et al.*, 2025) [18]. Furthermore, the amount of biologically effective UV radiation that reaches the ground is further reduced by high levels of atmospheric pollution, especially particulate matter that can deflect UV-B radiation (Rahman and Elmi, 2021) [45]. Despite theoretically favourable sun irradiation, these factors collectively decrease the cutaneous synthesis of vitamin D.

Dietary intake patterns compound the problem. Fatty fish, liver, egg yolk, and several fortified foods are the main natural dietary sources of vitamin D. Due to dietary preferences or financial limitations, regular consumption of these items is still restricted in many developing nations (Cashman, 2022) [11]. Additionally, there are significant variations in the amount of vitamin D consumed by the population due to the uneven implementation of fortification programs among nations. For instance, compared to areas where fortification regulations are either nonexistent or optional, obligatory fortification of dairy products in several parts of North America and Northern Europe has resulted in a comparatively higher average intake (Alnafisah *et al.*, 2024) [3]. As a result, people who mostly eat plant-based diets may have more difficulty getting enough vitamin D without supplements or fortification.

The health implications of widespread vitamin D deficiency extend beyond its classical role in calcium homeostasis and skeletal integrity. Severe deficiency has historically been linked to osteomalacia in adults and rickets in children (de Souza *et al.*, 2024) [15]. However, growing evidence suggests that vitamin D affects several physiological processes, such as cellular proliferation, endocrine function, and immunological modulation. Observational studies have linked low serum 25(OH)D concentrations to increased risk of osteoporosis, fractures, cardiovascular disease, metabolic disorders, and certain autoimmune conditions, although

causal relationships remain under active investigation (Pittas *et al.*, 2010) [44]. Concurrently, mechanistic research has shown vitamin D receptors in a variety of tissues, confirming the concept that vitamin D is a pleiotropic hormone involved in immune modulation and gene regulation (MacDonald, 1999; Carlberg, 2019) [10, 32].

Even with this growing awareness of vitamin D physiology, there is nonetheless substantial uncertainty over optimum serum 25(OH)D thresholds and recommended intake levels. Typically, cut-offs for deficiency and sufficiency range from 30 to 75 nmol/L, however different health organizations have different recommendations. However, there is still widespread acceptance that measures to raise population vitamin D levels are necessary and that severe deficiencies are undesirable.

In this regard, food-based strategies have attracted more attention as long-term, populationlevel interventions. Serum vitamin D concentrations can be efficiently raised by supplementation programs, but long-term adherence is frequently poor, especially in countries with limited resources. Although food fortification has a broader reach, it usually depends on centralized processing and regulations that might not be applied everywhere (Aghapour *et al.*, 2024) [1]. As a result, biofortification improving the nutrient content of foods during production or post-harvest processing has surfaced as a different approach that can include nutritional improvement straight into food supply chains (Li *et al.*, 2024) [31, 30].

**Edible Mushrooms as a Unique Non-Animal Source of Vitamin D**

Edible mushrooms occupy a distinctive niche within food-based strategies to improve vitamin D intake. Unlike most plant-derived foods, mushrooms contain substantial quantities of ergosterol, a key fungal membrane sterol that serves as the photochemical precursor of vitamin $D_2$.. As a result, when exposed to ultraviolet radiation, mushrooms act as a latent non-animal source of physiologically relevant vitamin D, addressing a fundamental constraint of diets high in plant-based or low in animal products. Mushrooms are extensively consumed across cultures and can be grown in very low-resource agricultural settings, making them a scalable and culturally flexible vehicle for dietary vitamin D delivery. Advancements in controlled UV-B irradiation technologies enable producers to reliably enhance vitamin $D_2$ concentrations to nutritionally meaningful levels, often without compromising sensory quality. This strengthens the feasibility of mushrooms as a practical food intervention.

Under natural growing conditions, sunlight-exposed wild mushrooms can accumulate significant levels of vitamin $D_2$,, often exceeding concentrations found in foods of animal origin typically consumed. On the other hand, even though ergosterol is present, commercially grown mushrooms are usually grown in regulated, dark settings to maximise yield and morphological consistency, resulting in a negligible baseline vitamin $D_2$ level (Mattila *et al.*, 2002) [35]. This contrast highlights how crucial UV exposure is in controlling the production of vitamin $D_2$ in mushrooms and serves as the theoretical foundation for post-harvest UV-B biofortification techniques.

From a compositional perspective, the ergosterol content, tissue structure, and water composition of commonly consumed mushroom species like *Agaricus bisporus* (button mushroom), *Pleurotus spp.* (oyster mushrooms), and *Lentinula edodes* (shiitake) vary, all of which affect their ability to produce vitamin $D_2$ when exposed to UV light. Although significant interspecies and intra-species heterogeneity has been reported, reported ergosterol concentrations generally fall between 2 and 10 mg per gram of dry weight. These variations are not only quantitative but also structural, since UV-B radiation penetration and dispersion inside mushroom tissues are influenced by cap morphology, gill density, and surface area-tovolume ratio. (Ben and Orsat, 2026) [6].

In addition to their ability to synthesise vitamin $D_2$, the nutritional value of mushrooms as a source of vitamin D depends on practical consumption habits. When expressed on a fresh weight basis, the high moisture content of fresh mushrooms (usually 85-95%) dilutes vitamin $D_2$ concentrations. Nevertheless, under ideal irradiation conditions, UV-treated mushrooms can still provide significant amounts of vitamin $D_2$ per serving, with reported levels ranging from 5 to over 20 µg per 100 g fresh weight (Tiwari *et al.*, 2022) [59]. When mushrooms are frequently consumed as part of mixed diets, these amounts are adequate to achieve or surpass recommended daily intakes in various dietary guidelines. (Stark *et al.*, 2024) [65].

The matrix in which vitamin $D_2$ is supplied in whole foods may affect its absorption and bioaccessibility. Dietary fibre, chitin, and other structural polysaccharides found in mushrooms may be able to influence the release of nutrients during digestion. Though there has been variation in response, recent *in vitro* digestion models and a small number of human studies indicate that vitamin $D_2$ from UV-exposed mushrooms is bioaccessible and capable of elevating serum 25(OH)D concentrations (Leung and Cheung, 2021) [29]. It nevertheless remains unclear how much co-ingestion, mastication, and cooking methods impact the absorption of dietary lipids. (Rodrigues *et al.*,2022) [46].

Mushrooms offer more than just vitamin D. They also contain B vitamins, selenium, and bioactive substances like β-glucans that have immunomodulatory characteristics. While these characteristics are not directly related to vitamin D metabolism, they may increase the nutritional value of mushrooms as part of a health-promoting diet. Importantly, integrating vitamin D biofortification with mushrooms' basic nutritional profile supports their status as multifunctional foods within dietary programs targeted at alleviating micronutrient deficiencies. (Moniruzzaman *et al.*, 2025) [39].

Despite these benefits, several constraints prevent mushrooms from being widely used as a primary dietary source of vitamin D. Mushrooms are not a staple food in many regions, and consumption levels vary significantly across populations (Ogwu *et al.*, 2025) [42]. Additionally, consumer awareness and regular intake may be hampered by variations in vitamin $D_2$ content resulting from processing and a lack of standardised labelling. The extent to which UV-treated mushrooms are promoted as functional foods is influenced by the regulatory frameworks governing vitamin D claims in foods (Tiwari *et al.*, 2021) [66].

**Ergosterol and Photochemical Conversion to Vitamin $D_2$**

The presence of ergosterol, a *Δδ,ß-sterol* that is the predominant structural sterol in fungal membranes, provides the biochemical basis for vitamin D biofortification based on mushrooms. The conjugated double-bond structure of ergosterol gives it photochemical reactivity, which is

essential for the production of vitamin $D_2$,, making it more than just a compositional equivalent of cholesterol (Choy *et al.*, 2023) [12]. Ergosterol is distributed differently across tissues, including caps, gills, and stipes, and is concentrated in the membranes of intracellular organelles and the plasma membrane of edible mushrooms. This tissue-level variability has practical implications since photoconversion efficiency is determined by the percentage of ergosterol that is both physically accessible to UV photons and capable of undergoing subsequent thermal isomerisation (Thakur *et al.*, 2024) [58].

The conversion of ergosterol to vitamin $D_2$ follows a photochemical pathway mechanistically analogous to the cutaneous synthesis of vitamin $D_3$ from 7-dehydrocholesterol in human skin. Upon exposure to UV-B radiation, ergosterol undergoes a photolytic cleavage of the B-ring (specifically the C9-C10 bond), yielding pre-vitamin $D_2$ through a conrotatory electrocyclic reaction. Although the effective action spectrum is dependent on the sterol microenvironment and matrix optical properties, this reaction is predominantly driven by photon absorption in the UV-B range, with maximal absorption usually recorded at 280-300 nm. Thermodynamically unstable, the resultant pre-vitamin $D_2$ spontaneously experiences a temperature-dependent [1, 7]-sigmatropic hydrogen shift to become vitamin $D_2$ (Sommer *et al.*, 2023) [53].

Nevertheless, neither linearity nor quantitative efficiency characterize the photoconversion route. Pre-vitamin $D_2$ exists in equilibrium with several photoproducts, which is a crucial mechanistic subtlety. Lumisterol$_2$ and *tachysterol*$_2$ are examples of physiologically inactive sterol derivatives that can be produced by additional photochemical rearrangements brought on by prolonged UV exposure (Sommer *et al.*, 2024) [52]. A non-monotonic dose-response curve, where vitamin D2 concentration rises initially but may plateau or decline at high UV doses due to photodegradation and photoproduct accumulation, is produced by these competing reactions, which impose an intrinsic ceiling on achievable vitamin D2 yield under prolonged irradiation. (Pedrali *et al.*, 2020) [43].

The physical condition of ergosterol in mushroom tissues affects this equilibrium between competing photochemical side reactions and productive synthesis. When compared to crystalline ergosterol or extracted sterol standards, ergosterol embedded in lipid bilayers may show different absorption properties (Juarez-Contreras *et al.*, 2025) [25]. Both photon absorption efficiency and the likelihood of ring-opening processes can be influenced by the microenvironment of the sterol, which includes lipid composition, membrane fluidity, hydration, and protein interaction. Furthermore, secondary degradation pathways may be impacted by the presence of endogenous antioxidants or Reactive Oxygen Species (ROS) produced after UV exposure, albeit there is still little data and less mechanistic research in fungal matrices (Menoni *et al.*, 2025) [37].

Optical penetration depth is another important characteristic that sets mushroom systems apart from human cutaneous synthesis. UV-B radiation has limited penetration into hydrated biological tissues due to scattering and absorption by water and organic compounds. As a result, photoconversion is most efficient at or near the surface of mushroom tissues, particularly in gill structures where surface area is large and tissue thickness is low. This explains why intact mushrooms irradiated from the cap surface frequently exhibit significantly lower vitamin $D_2$ accumulation than split mushrooms or mushrooms irradiated gill-side-up (Nölle *et al.*, 2017) [41]. Thus, photon transmission to ergosterol-rich microdomains and ergosterol concentration both limit vitamin D2 yield.

Thermal isomerisation of pre-vitamin $D_2$ to vitamin $D_2$ is also a rate-limiting process. UV exposure triggers ring opening, but converting to stable vitamin $D_2$ requires sufficient heat energy. This procedure is more efficient at moderate temperatures, indicating that postirradiation holding conditions (time and temperature) can impact final vitamin $D_2$ levels. Some studies show that vitamin $D_2$ concentrations increase after irradiation during storage at ambient temperatures, indicating continuous isomerisation (Li, 2024) [31, 30]. Rapid cooling can hinder photoproduct conversion and dispersion, making it difficult to quantify vitamin $D_2$ soon after UV exposure (Cardwell *et al.*, 2023) [9].

The ergosterol-to-vitamin $D_2$ route in mushrooms is a controlled photobiochemical system that is regulated by competing photoproduct synthesis, tissue optics, and sterol chemistry. In addition to highlighting inherent yield constraints and sources of variability, this mechanistic approach offers the scientific justification for UV-B irradiation as a biofortification technique.

**UV-B Irradiation for Vitamin D Biofortification**

The technical objective of UV-B irradiation is to minimize oxidative degradation and competitive photoproduct production while optimizing ergosterol conversion to vitamin $D_2$.. Wavelength distribution, dose (irradiance × exposure time), exposure geometry, and postirradiation thermal equilibration are among the irradiation characteristics that must be optimized in practice (Ben and Orsat, 2026) [6]. Research consistently shows that the production of vitamin $D_2$ is dose-dependent at low to moderate UV exposure levels, usually growing rapidly in the early phases of irradiation before plateauing. Vitamin $D_2$ may either stagnate or decrease at high dosages due to direct photodegradation or conversion into secondary photoproducts. Therefore, beyond a certain point, "more $UV^n$ does not equate to "more vitamin

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UV-B is often preferred because it aligns with the natural action spectrum for ergosterol conversion and has a lesser propensity to cause severe tissue damage, even though both UV-B and UV-C radiation can trigger vitamin $D_2$

production. Although UV-C can effectively produce vitamin $D_2$ in surface layers, it can also cause oxidative changes, discolouration, and possible quality degradation. Furthermore, because of its antibacterial use and possible consumer concerns, UV-C irradiation is frequently controlled differently. In contrast, ergosterol absorption is less at longer wavelengths, making UV-A radiation noticeably less effective. Consequently, UV-B lamps or broadband UV sources intended to approximate the effective ergosterol absorption spectrum are used in the majority of food-grade systems. (Neill *et al.*, 2023) [40].

A common methodological problem in the literature is the inconsistent reporting of UV dosage. While some research offers lamp power without measuring surface irradiance at the sample plane, others describe exposure duration without mentioning irradiance. Nominal lamp power is a poor surrogate for biologically effective UV dose since irradiance is highly dependent on lamp distance, lamp ageing, reflector design, and ambient temperature. Radiometers are increasingly used in high-quality research to measure irradiance in $W/m^2$ or $mW/cm^2$ and provide total dosage in $J/m^2$. Cross-study comparison is unreliable in the absence of standardised dose reporting, which contributes to apparent contradictions in reported vitamin $D_2$ outputs. (Wu *et al.*, 2014) [62].

The geometry of irradiation is similarly important. Self-shading is produced by the intricate three-dimensional structures of mushrooms, especially in intact specimens. When mushrooms are exposed to cap-up radiation, gill tissue which frequently has a high ergosterol density and surface area is partially shielded. Research consistently demonstrates that employing fragmented tissues, chopping mushrooms, or orienting them gill-side up significantly improves vitamin D2 accumulation per unit dose. This implies that photon access, rather than precursor supply, limits the efficiency of irradiation. From an industrial perspective, slicing boosts yield but may shorten shelf life because of increased surface exposure and moisture loss, illustrating a trade-off between product quality and biofortification efficiency. (Nölle *et al.*, 2017) [41].

Beyond UV-B, emerging approaches incorporate pulsed UV light, light-emitting diodes (UV-LEDs), and hybrid UV-visible systems. Pulsed UV systems deliver high-intensity bursts that may increase conversion rates while reducing total processing time, though the effect on photoproduct formation remains under-characterised (Kalaras *et al.*, 2012) [26]. UV-LED technology offers wavelength specificity, lower heat output, and potential energy efficiency advantages. However, the narrow emission spectrum may require careful selection to match ergosterol absorption maxima, and current UV-LED systems can be cost-limiting at an industrial scale. Still, UV-LEDs represent a likely future direction due to their tunability and integration potential into automated processing lines (Hidalgo-Sanz *et al.*, 2023) [22]

Quality retention is an additional constraint. Browning, altered aroma profiles, and oxidative lipid alterations can be caused by UV radiation, especially at high levels. Despite having a low fat content, mushrooms have taste precursors and unsaturated lipids that could be oxidised. Due to variations in species, dosage, and storage circumstances, some studies report negligible sensory changes at nutritionally efficient UV dosages, while others notice noticeable discolouration or texture changes. This variation emphasises how crucial consumer and sensory testing are in addition to biochemical quantification when assessing practicality. (Wu *et al.*, 2016) [63]

Overall, UV-B irradiation is a theoretically feasible and scalable technique for vitamin $D_2$ biofortification; yet, its effectiveness relies on exact control over dose, exposure geometry, and post-treatment conditions. Although the literature shows considerable inter-study variation caused by inconsistent methodology and species-specific variances, it also firmly supports the viability of reaching nutritionally important vitamin $D_2$ levels. The following section on factors influencing vitamin $D_2$ accumulation in mushrooms discusses the biological and processing determinants of vitamin $D_2$ generation in further detail.

## Factors Influencing Vitamin $D_2$ Formation in Mushrooms

A complex interplay between precursor availability (ergosterol content), photon delivery (UV-B exposure conditions), tissue optics (penetration and scattering), and post-photochemical kinetics (thermal isomerization and competing photoproduct formation) determines the effectiveness of vitamin $D_2$ biofortification in mushrooms. Variability in vitamin $D_2$ yield across studies suggests that irradiation dose alone is not adequate to predict biofortification outcomes, even if UV-B irradiation provides the initial energy for ergosterol photolysis. Rather, it is better to think of vitamin $D_2$ accumulation as an emergent characteristic of fungal physiology and processing geometry, limited by both external technical constraints and intrinsic photochemical ceilings.

### Species and Strain Differences

Mushroom species are among the most powerful biological determinants. Due to its worldwide commercial domination, *Agaricus bisporus* has been studied the most, other cultivated mushrooms, such as *Pleurotus spp.*, *Lentinula edodes*, and *Flammulina velutipes*, exhibit different tissue morphologies and sterol profiles (Taofiq *et al.*, 2017) [57]. These variations impact the concentration of total ergosterol as well as its spatial distribution and membrane attachment status. Under identical UV exposures, several comparison studies claim higher vitamin $D_2$ outputs in oyster mushrooms compared to button mushrooms. This could be due to thinner tissues and higher effective surface exposure rather than essentially higher ergosterol levels. On the other hand, because of their denser tissue structure, shiitake mushrooms may have more variability despite typically having small yields (Saini *et al.*, 2021) [48].

Strain-level variations can be significant even within a single species. Fungal metabolic processes that are sensitive to temperature, growth stage, and substrate composition control ergosterol production. Variability in baseline ergosterol pools available for photoconversion may result from cultivation techniques targeted for yield that unintentionally affect sterol metabolism. This presents a sometimes disregarded upstream factor: biofortification efficiency starts during culture rather than irradiation, indicating the possibility of incorporating breeding or metabolic selection into the production of commercial vitamin D mushrooms (Song *et al.*, 2025) [54].

### Tissue Morphology and Surface Area Constraints

Mushroom form has a significant effect on photon accessibility. UV-B penetration into hydrated tissues is limited, hence surface area-to-volume ratio is an important

factor. Gill tissues contain a vast surface area and are often sterol-rich, but are partially shaded in whole mushrooms. Studies indicate that slicing mushrooms increases vitamin $D_2$ levels per unit dose, suggesting that conversion is limited by optical access rather than ergosterol deficiency. Slicing, on the other hand, increases water loss while also hastening enzymatic browning and microbiological deterioration, making commercial use difficult. Thus, morphological modification creates a clear trade-off between biofortification yield and shelf-life stability (Song *et al.*, 2025) [54].

**UV Dose, Intensity, and Exposure Time Interactions**

UV dosage is commonly defined as irradiance multiplied by exposure time, however intensity and time are not interchangeable since photoproduct dynamics are nonlinear. Highintensity, short-duration exposure can result in different vitamin $D_2$: photoproduct ratios than low-intensity, long-duration exposure, even if the total dose is the same. This is compatible with photochemical reaction networks, where competing routes have varying quantum yields and rely on intermediate accumulation. Few research have directly tested intensity-time reciprocity, indicating a large mechanistic gap. If reciprocity fails, industrial systems must optimize intensity profiles rather than relying on dose-equivalence assumptions. (Fitzner *et al.*, 2024) [21].

**Temperature and Post-Irradiation Holding**

Temperature affects the conversion of pre-vitamin $D_2$ to vitamin $D_2$, as well as the degradation of vitamin $D_2$ after UV exposure. Research suggests that vitamin $D_2$ levels may continue to rise following irradiation and storage at room temperature due to isomerisation. Immediate freezing can "freeze" the reaction mixture, resulting in reduced vitamin $D_2$ levels unless samples are allowed to equilibrate. Vitamin $D_2$ quantification is sensitive to sample handling time between irradiation and extraction, which may contribute to heterogeneity among studies. Standardisation of post-irradiation equilibration settings is thus required for consistent findings (Kamal *et al.*, 2025) [27].

**Methodological Variability**

Inconsistent analytical methods are a non-trivial source of apparent variability. Fresh weight, dry weight, or serving basis can all be used to report vitamin $D_2$; the conversion between these measures is dependent on moisture content. Systematic variations between laboratories can also result from variations in calibration standards, saponification procedures, and extraction efficiency. If photoproducts co-elute under insufficient chromatographic resolution, certain studies may overstate vitamin $D_2$. These analytical uncertainties make mechanistic interpretation more difficult and highlight the necessity of standardized techniques (McGinty and Phillips, 2024) [36].

In conclusion, biological (species, tissue composition), physical (surface area), and processing (dose, intensity, temperature) factors interact to regulate the production of vitamin $D_2$ in UVtreated mushrooms. High vitamin $D_2$ yields are feasible, according to the literature, but it also shows a complicated optimization landscape where advances in conversion efficiency may come at the expense of shelf life, sensory quality, or process scalability. When post-harvest processing and storage are taken into account, these limitations become very important since they affect whether vitamin $D_2$ produced during irradiation is preserved via distribution and cooking.

**Post-Harvest Processing and Stability of Vitamin $D_2$**

In the end, retention throughout post-harvest handling, storage, distribution, and culinary preparation determines the practical nutritional value of UV-biofortified mushrooms rather than peak vitamin $D_2$ concentrations right after irradiation. Due to its conjugated triene system and chemical classification as a secosteroid, vitamin $D_2$ is vulnerable to deterioration by photooxidation, thermal isomerization, and interaction with reactive oxygen species. Therefore, a key translational bottleneck is the stability of vitamin $D_2$ in mushrooms: high conversion yields are meaningless if the molecule significantly degrades prior to consumption. Therefore, stability should not be viewed as a downstream quality-control concern but rather as an essential component of biofortification optimization (Pedrali *et al.*, 2020) [43].

**Storage Conditions and Shelf-Life Retention**

Vitamin $D_2$ produced in mushrooms is comparatively stable while stored in a refrigerator, especially when shielded from light. Vitamin $D_2$ retention frequently stays high for at least 5- 10 days under normal cold-chain conditions (4-8 °C), while reported degradation rates differ significantly between investigations (Cardwell *et al.*, 2023) [9]. Variability probably reflects variations in moisture loss, microbial spoilage processes, and packaging environment (oxygen availability), all of which may have an indirect impact on oxidative chemistry (Ban *et al.*, 2014) [5]. Crucially, storage stability depends on both the physical integrity of mushroom tissues and the microenvironment produced by packaging systems. It should not be seen as an intrinsic chemical feature alone.

One of the main factors influencing degradation during storage is exposure to light. When exposed to UV or visible light, vitamin $D_2$ can undergo photochemical rearrangement, resulting in oxidative byproducts and inactive isomers. Therefore, vitamin D2 losses may be exacerbated by transparent packaging that is displayed in store illumination. This leads to a practical paradox: whereas UV treatment is intended to produce vitamin $D_2$,, uncontrolled light exposure after treatment may hasten its breakdown. For vitamin D2 content to be preserved during retail distribution, packaging solutions that minimise light transmission such as opaque films or UV-blocking materials may be required. (Marçal *et al.*, 2021) [34].

**Thermal Processing and Cooking Losses**

Cooking is an unavoidable variable in real-world dietary intake and a critical determinant of vitamin $D_2$ retention. Most cultures rarely eat mushrooms raw; instead, they are usually sautéed, boiled, grilled, baked, or added to other meals. Vitamin $D_2$ is slightly heat-stable when compared to a number of labile vitamins, although it is vulnerable to thermal deterioration, particularly when heated for prolonged periods of time or in the presence of oxygen. Depending on the cooking method and time, reported retention after cooking varies greatly, from negligible loss to reductions exceeding 30-50%. While boiling may result in losses owing to leaking into cooking water in addition to thermal breakdown, frying and grilling may preserve vitamin $D_2$ relatively well because of shorter exposure times. (Cardwell *et al.*, 2023) [9]

**Drying and Dehydration Processes**

Drying is a common industrial and home preservation technique for mushrooms, and it may be used to increase nutrient density per gram while stabilizing vitamin $D_2$.. Dehydration lowers water activity and may restrict microbial and enzymatic activities that indirectly cause oxidation. Freeze-drying, vacuum drying, hot-air drying, and sun-drying have all been studied; freeze-drying generally preserves vitamin $D_2$ more well because of its low temperature. If exposure lengths are extended or temperatures are high, hot-air drying may cause vitamin $D_2$ breakdown. Increased UV exposure from sun-drying may both boost the production of vitamin $D_2$ and encourage photodegradation, depending on the duration and intensity of exposure. Therefore, depending on how it is handled, drying can serve as both a fortification and a degrading process (Pedrali *et al.*, 2020) [43]

An important but least studied question is whether dried mushrooms retain their photochemical reactivity. Dried mushrooms may still produce more vitamin $D_2$ after being exposed to UV light because ergosterol is still present after dehydration. This could allow for multi-stage processing: controlled UV irradiation at the final packaging stage after drying for preservation. However, because of changed optical characteristics and sterol crystallization, the conversion efficiency in dried matrices may vary, necessitating specialized mechanistic research (Jiang *et al.*, 2020) [24].

**Freezing and Long-Term Storage**

Mushrooms are frequently frozen for long-term storage, although there is still conflicting and scant data on vitamin D2 stability in cold environments. Low temperatures should, in theory, promote high retention by slowing oxidative destruction. Freeze-thaw cycles, however, have the potential to affect the stability of vitamin $D_2$ after thawing by disrupting tissue structure, increasing oxygen diffusion, and encouraging moisture migration. Furthermore, vitamin $D_2$ may partition differentially in damaged membranes, which could have an impact on observed concentrations and extraction efficiency. Stability evaluations under frozen storage must so closely monitor the freeze-thaw history and incorporate extraction recovery confirmation. (Sławińska *et al.*, 2016) [51].

**Processing into Mushroom Powders and Food Ingredients**

From the perspective of food fortification, the use of mushrooms in powders, seasoning blends, protein substitutes, and composite food products is growing. When UV-treated mushrooms are ground into powder, their surface area increases significantly, which may make them more vulnerable to oxidation. However, powders offer stability benefits when packaged in inert environments. Light exposure, moisture control, and packing oxygen levels all affect the net stability result. The "whole food" benefit of mushroom-based biofortification may be compromised by encapsulation techniques like microencapsulation of vitamin $D_2$ fractions or mushroom extracts, which move the intervention closer to conventional fortification (De and Goswami, 2022) [16].

In conclusion, post-harvest processing has a significant impact on the actual nutritional value of UV-biofortified mushrooms. While cooking effects vary depending on method and dish composition, vitamin $D_2$ retention appears to be generally compatible with refrigerated storage under light-protective packaging. Although they present new oxidative risks, drying and powder manufacturing present opportunities for nutrient-dense vitamin $D_2$ compounds. All of these results suggest that mass-balance methods, uniform storage conditions, and photoproduct profiling should be used to assess vitamin $D_2$ stability.

**Bioavailability and Health Effects of Mushroom-Derived Vitamin $D_2$**

The translational value of UV-B-biofortified mushrooms ultimately depends on whether mushroom-derived vitamin $D_2$ is bioavailable, metabolically functional, and capable of improving vitamin D status in humans at realistic dietary intake levels. Mechanistic plausibility alone ergosterol conversion to ergocalciferol does not guarantee nutritional efficacy, because vitamin D activity depends on intestinal absorption, transport via vitamin D binding protein (DBP), hepatic conversion to 25-hydroxyvitamin $D_2$ [25 (OH) $D_2$] ·, renal hydroxylation to the hormonally active 1,25-dihydroxyvitamin $D_2$, and downstream receptor-mediated signaling. Thus, bioavailability should be evaluated not merely as absorption but as the integrated capacity of mushroom-derived vitamin $D_2$ to raise circulating total 25(OH)D concentrations and support biological endpoints relevant to skeletal and extra-skeletal health (Dong *et al.*, 2020) [17].

**Bioaccessibility and Intestinal Absorption: Mechanistic Considerations**

Vitamin $D_2$ is fat-soluble and requires incorporation into mixed micelles during digestion for efficient intestinal uptake. Therefore, co-ingestion with dietary lipids, bile acid availability, and gastrointestinal emulsification dynamics are expected to modulate absorption efficiency. Although mushrooms are naturally low in fat, they contain complex polysaccharides and fiber, such as chitin and β-glucans, which may affect micellarization and digestion kinetics (Steinbauer *et al.*, 2025) [56]. Although the bioaccessible fraction can vary significantly depending on the cooking method and whether the mushrooms are consumed with fat-containing meals, *in vitro* digestion studies employing standardized INFOGEST-type protocols generally show that vitamin $D_2$ in UV-treated mushrooms is bioaccessible (Brodkorb *et al.*, 2019) [7]. These results are consistent with recognized lipid-soluble vitamin physiology, but they highlight the possibility that the context of a meal may have a greater impact on vitamin $D_2$ provided by mushrooms than on vitamin D delivered through oil-based supplements (Yan *et al.*, 2024) [64].

**Human Evidence:** Effects on Serum 25(OH)D

Human intervention studies that measure changes in serum 25(OH)D provide the strongest evidence for the bioavailability of vitamin $D_2$ derived from mushrooms. Consuming UVexposed mushrooms has been demonstrated in numerous controlled studies to raise serum 25(OH)$D_2$ concentrations and, frequently, total serum 25(OH)D. This indicates that canonical hydroxylation mechanisms are used for the absorption and metabolism of vitamin $D_2$ from mushrooms. The size and consistency of trial results, however, differ due to differences in baseline deficiency status, comparator interventions (placebo, vitamin $D_2$

supplement, or vitamin $D_3$ supplement), mushroom preparation (fresh *vs.* dried *vs.* cooked), and dosage approach (daily *vs.* weekly) (ClinicalTrials.gov. (2023) [13].

A recurring finding is that increases in serum 25 (OH) $D_2$ may coincide with reductions in serum 25(OH)$D_3$, resulting in smaller net increases in total 25(OH)D than expected from the administered dose. This phenomenon, which has been seen in vitamin $D_2$ supplementation experiments, could be the result of rapid catabolism of vitamin $D_3$ metabolites, altered hepatic hydroxylation dynamics, or competitive binding interactions at DBP. It is still debatable whether this effect is clinically significant or biologically significant. While some metaanalyses show similar efficacy at an appropriate dose and throughout shorter intervention periods, others imply vitamin $D_2$ is less successful than vitamin $D_3$ at sustaining total 25(OH)D over time. (Mohammad Alayed Albarri *et al.*, 2022) [38].

**Dose-Response and Realistic Intake Scenarios**

A critical translational question is whether typical mushroom consumption patterns can deliver clinically meaningful vitamin $D_2$ doses. Under optimal processing, UV-treated mushrooms can have more than 10-20 µg of vitamin $D_2$ per 100 g of fresh weight. Daily intakes in numerous clinical studies range from 5 to 15 µg of vitamin $D_2$, which is in accordance with several national recommendations' suggested dietary allowances. Increases in serum total 25(OH)D under these circumstances are frequently slight but noteworthy, particularly in people with poor baseline status. Higher therapeutic dosages may be necessary in populations with severe deficiencies (<25-30 nmol/L), and mushrooms by themselves might not be adequate unless consumption is high or products are made into concentrated powders or extracts (Ferraris *et al.*, 2025) [20].

The strongest case for mushrooms is therefore not as a replacement for supplementation in clinical deficiency treatment, but as a population-level dietary strategy to shift mean vitamin D status upward and reduce prevalence of insufficiency. This aligns with fortification logic: small daily increments distributed widely can yield meaningful public health impact even if individual-level effects appear modest

**Safety and Upper Intake Considerations**

Vitamin D toxicity is rare and generally associated with excessive supplement intake rather than food sources. When consumed as food, UV-treated mushrooms usually offer vitamin $D_2$ doses within safe dosage ranges, even when they are significantly enriched. However, product standardisation is crucial since uncontrolled UV exposure may result in extremely variable vitamin $D_2$ concentrations, making it more difficult to estimate dietary intake. This is especially important if mushroom powders are added to several processed items, which could result in cumulative consumption. Therefore, in addition to toxicity, safety evaluation should take batchto-batch uniformity and labelling accuracy into account. (Won *et al.*, 2018) [61].

The conclusion that vitamin $D_2$ derived from mushrooms is accessible and can enhance vitamin D status is supported by the existing data. Nonetheless, a number of translational and mechanical ambiguities still exist. There are not many well-powered studies that directly compare UV-mushrooms with meals supplemented with vitamin D3 under similar dosage schedules. Additionally, nothing is known about how gut microbiota interactions, cooking techniques, and meal fat content affect the kinetics of vitamin $D_2$ absorption.

In synthesis, UV-biofortified mushrooms offer a physiologically useful form of vitamin $D_2$ that can increase human circulation vitamin D metabolites. Clinical outcome data are still few, but biochemical endpoints (serum 25 (OH) $D_2$ and total 25(OH)D) have the greatest evidence basis. Although concentrated mushroom-based ingredients may broaden this reach, their most likely public health role is in sustainable dietary enrichment measures rather than therapeutic correction of severe insufficiency.

**Industrial Applications and Food Fortification**

The ability of UV-B-biofortified mushrooms to deliver vitamin $D_2$ through an endogenous, scalable, and possibly cost-effective approach compatible with current mushroom supply chains is what gives them industrial value. In contrast to chemical fortification, which necessitates the addition of purified vitamin D and strict dispersion control, UV-based biofortification produces vitamin $D_2$ directly within the tissues of mushrooms, simplifying formulation and allowing for "clean-label" positioning in markets where consumers are still sceptical of additives.

Continuous conveyor-based processing equipment that can handle huge volumes with regulated exposure time and lamp intensity have replaced batch irradiation systems in commercial deployment. The best industrial viability occurs when irradiation is incorporated right after harvest, before packaging, allowing for consistent vitamin D2 content and centralized quality control. Engineering limitations are still difficult to overcome, though, as non-uniform exposure brought on by stacking density, self-shading, and uneven morphology can result in significant within-batch variability. Tumbling systems, dual-sided irradiation, reflective chambers, and optimum layer thickness are some of the suggested solutions, however they raise the complexity and energy consumption of the equipment (Hidalgo-Sanz *et al.*, 2023) [22].

Vitamin $D_2$. -enriched mushroom powders are a very appealing industrial additive in addition to fresh mushrooms. Drying increases shelf life, concentrates vitamin $D_2$ per unit mass, and makes it easier to incorporate into a variety of food matrices, such as soups, noodles, baked goods, snack spices, and meat substitutes. These powders support population-level intervention logic by enabling the fortification of staple foods without changing consumer eating habits (Anusha *et al.*, 2022) [4]. However, the manufacture of powder creates additional concerns of oxidative degradation and batch inconsistency, requiring validated stability testing and oxygen-controlled packing (Pedrali *et al.*, 2020) [43].

Industrial uptake is significantly influenced by regulatory classification. UV-treated mushrooms are classified as conventional foods with altered nutrient composition in some countries, while they are controlled as novel foods or need special labeling in others. International trade and widespread adoption are complicated by the lack of harmonization. Furthermore, there is a strong incentive for consistent analytical verification because eligibility for health claims is contingent upon confirmed vitamin D content stability during shelf-life and compliance with fortification restrictions (EFSA,2024) [19].

With all factors taken into account, industrial deployment is theoretically feasible; nonetheless, long-term success hinges on process standardization, accurate quantification of vitamin $D_2$, sensory neutrality, and regulatory clarity. Future industrial initiatives will probably focus on automated dose monitoring, UV-LED systems for wavelength precision, and incorporating vitamin $D_2$ compounds obtained from mushrooms into larger fortification projects aimed at vulnerable populations.

**Future Research Directions**

UV-B biofortification of mushrooms is nevertheless constrained by unresolved biochemical, technical, and translational constraints despite high mechanistic plausibility and mounting evidence for human bioavailability. Standardizing UV dose reporting, sample orientation procedures, and post-irradiation equilibration conditions should be the top priorities for future research in order to reduce inter-study heterogeneity. Without harmonization, industrial scaling still depends on empirical optimization rather than predictive process design, and comparisons between studies remain poor.

One untapped area is optical modeling of UV penetration in mushroom tissues. Current irradiation optimization is mostly heuristic, despite the fact that photon delivery is greatly influenced by mushroom morphology (Hsieh *et al.*, 2020) [23]. Future studies should estimate vitamin $D_2$ synthesis in relation to geometry, moisture, and pigmentation by combining radiative transfer models with observations of tissue optical properties (absorption/scattering coefficients). This type of predictive modeling could save energy costs and expedite process design.

Although intriguing, the evidence regarding bioavailability in terms of nutrients is currently lacking. Particularly in high-risk populations (elderly, pregnant women, obese people, malabsorption disorders), well-powered randomized trials comparing UV-mushrooms with vitamin D3-fortified meals are required (Brown *et al.*, 2025) [8]. Under realistic dietary settings, stable isotope tracer experiments would shed light on the absorption kinetics, tissue distribution, and metabolic turnover of vitamin $D_2$ obtained from mushrooms.

Systematic research is also needed for food matrix effects. Vitamin $D_2$ bioaccessibility may be greatly impacted by cooking techniques, co-ingestion with lipids, and inclusion into composite foods, although the available results are inconsistent. Intestinal transport assays, such as *Caco-2* uptake and chylomicron secretion, could be integrated with standardized INFOGEST digestive models to offer mechanistic clarity connecting processing to absorption. (Saini *et al.*, 2025) [47].

Lastly, in order to convert biochemical innovation into population benefit, implementation science is required. Consumer acceptability, cost-effectiveness, integration of fortification policies, and supply-chain viability in low-resource environments should all be studied. UVbiofortified mushrooms run the risk of staying a specialized functional food rather than a scalable nutritional intervention in the absence of these assessments.

**Conclusion**

UV-B biofortification of edible mushrooms is a scientifically sound example of using endogenous food biochemistry to combat a prevalent micronutrient deficiency. The mechanism is well known: ergosterol, which is abundant in fungal membranes, is photolyzed by UV-B light to produce pre-vitamin $D_2$, which is then isomerized into ergocalciferol by thermal means. However, competing photoproduct processes (*lumisterol₂*, *tachysterol₂*) and tissue-level optical constraints that limit UV penetration naturally limit the conversion. The plateauing of vitamin $D_2$ yields at high doses is explained by these mechanistic ceilings, which also emphasize the need for photon delivery efficiency to be prioritized over exposure intensity in irradiation optimization.

Industrial feasibility is strongly supported. Scalable integration into mushroom manufacturing chains is made possible by conveyor-based UV systems, enhanced irradiation geometry, and new UV-LED platforms. However, morphological variation, shading effects, and variable UV dosage reporting across studies continue to pose challenges to reliable vitamin $D_2$ output. Standardized irradiation procedures, radiometric validation, and quality-control frameworks that guarantee consistent vitamin $D_2$ concentrations throughout shelf life are consequently essential for translation.

Critically, bioavailability evidence indicates that mushroom-derived vitamin $D_2$ is nutritionally functional. Increases in serum 25 (OH) $D_2$ and frequently total 25(OH)D are observed in human studies after consuming UV-exposed mushrooms, indicating their ability to improve vitamin D status at reasonable intake levels. However, there is still no data for clinical outcomes other than biochemical markers, and comparative efficacy in relation to vitamin D3 is still up for debate. Therefore, using UV-biofortified mushrooms as a food-based technique to increase population vitamin D distributions rather than as a stand-alone treatment intervention for severe deficiency is the most justifiable public health function.

Future progress requires mechanistic profiling of photoproduct networks, predictive optical modeling, standardized analytical protocols, and well-powered comparative clinical trials. Without these, variability in reported yields and uncertainty in long-term efficacy will continue to limit policy-level adoption. Overall, UV-B-biofortified mushrooms offer a credible, scalable, and mechanistically grounded approach to dietary vitamin D enhancement, with strong translational promise provided that process standardization and evidence integration keep pace with industrial expansion.

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