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Research Article

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Innovative Sun-Activated Vitamin D and UV-B Enhancement in Mushrooms for Smart Food Fortification and Functional Nutrition

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A B S T R A C T

This study evaluated a sustainable biofortification approach to enhance vitamin D_2 content in edible mushrooms and to translate this enhancement into acceptable functional food products. Fresh and dried mushrooms (*Agaricus bisporus*) were exposed to controlled natural sunlight at different times of day and to calibrated UV-B irradiation to optimize the photoconversion of ergosterol to vitamin D_2. Environmental parameters, including UV-B intensity, temperature, and humidity, were monitored to identify optimal exposure conditions. Both sunlight and UV-B treatments significantly increased vitamin D_2 levels compared with dark-dried controls (0.4 µg/g). Maximum enrichment was achieved under midday sunlight (up to 6.0 µg/g in fresh slices and 9.0 µg/g in ground dried samples) and following 45 min of UV-B exposure (up to 12.0 µg/g in ground samples), highlighting the importance of UV dose, exposure duration, and surface area. Excessive exposure led to reduced vitamin D_2 levels, indicating photodegradation beyond an optimal threshold. Protein content remained unchanged across treatments (~25.5–26.2%), confirming preservation of nutritional quality. The vitamin D–enriched mushroom powder was incorporated into instant noodle seasoning at different inclusion levels and evaluated using a 9-point hedonic sensory scale. All formulations exceeded the acceptance threshold (≥7), with high scores for taste, overall acceptability, and versatility, and a preference for higher inclusion levels. Overall, the findings demonstrate that optimized sunlight and UV-B exposure can produce nutritionally stable, vitamin D-rich mushroom powders with excellent consumer acceptability, supporting their potential as a scalable, plant-based strategy to address dietary vitamin D insufficiency.

INTRODUCTION

Vitamin D deficiency is a widespread public-health issue linked to impaired calcium homeostasis, poor bone health, and increased risk of fractures, as well as adverse effects on immune and metabolic function (Liu *et al.*, 2025). Traditional dietary sources are limited, prompting interest in sustainable, plant-based strategies to increase population vitamin D intake. One promising approach is the photochemical conversion of ergosterol to vitamin D_2 in edible mushrooms through post-harvest sunlight or calibrated UV-B exposure, which can substantially elevate vitamin D_2 content while preserving other nutritional and sensory properties (Williams, 2024).

Edible mushrooms are also rich in bioactive compounds, including polysaccharides, phenolics, proteins, and umami-active molecules such as free glutamic acid and 5′-nucleotides, which enhance palatability and support dietary adherence in plant-based or reduced-salt formulations (Huang *et al.*, 2023). Incorporating vitamin-D-enriched mushroom powders into staple foods like noodles provides a

practical fortification strategy, achieving sensory acceptance at 10-20% substitution while improving micronutrient content and dietary fiber (Cashman, 2024).

The functional and sensory qualities of mushroom powders depend on post-harvest handling and storage. Dry powders are shelf-stable due to low water activity, but rehydration or inclusion in prepared foods accelerates degradation of both vitamin D_2 and umami compounds. Understanding the "countdown to consumption" the temporal window during which fortified powders retain maximal nutritional and sensory quality is therefore essential for food safety and efficacy (Jacob *et al.*, 2025).

The critical roles of vitamin K and its interaction with vitamin D in regulating calcium metabolism and maintaining bone health. Vitamin K is essential for the γ-carboxylation of vitamin K-dependent proteins, notably osteocalcin and matrix Gla protein, which facilitate calcium incorporation into bone and inhibit ectopic calcification in soft tissues. Evidence from experimental studies and human observational and interventional trials indicates that poor vitamin K status is associated with increased undercarboxylated osteocalcin, reduced bone mineral density, and higher fracture risk, particularly in older adults (Xiao *et al.*, 2021).

While vitamin D enhances intestinal calcium absorption and supports systemic calcium homeostasis, vitamin K ensures appropriate calcium utilization at the skeletal level. Studies assessing combined supplementation demonstrate more favorable effects on bone turnover markers, maintenance of bone mineral density, and suppression of vascular calcification markers compared with either vitamin alone, with vitamin K_2 forms such as menaquinone-7 showing particular efficacy. Although fracture outcome data remain inconsistent, the overall evidence supports a synergistic role of vitamins K and D in skeletal health, suggesting that optimizing both nutrients may be more effective than single-vitamin strategies for preserving bone integrity and preventing calcium-related disorders (Aaseth *et al.*, 2024).

The aim of this study was to optimize sunlight and UV-B exposure conditions to enhance vitamin D_2 content in edible mushrooms and to evaluate the nutritional stability and sensory acceptability of the resulting vitamin D-enriched mushroom powder when applied as a smart, sustainable fortification ingredient in functional food products.

MATERIALS AND METHODS

Sampling and treatments

Fresh edible white button mushrooms (*Agaricus bisporus*) were obtained from Al Mansoura Mushroom Production Company and selected for uniform size, color, and maturity, free from visible defects or microbial spoilage. Mushrooms were thinly sliced and assigned to three experimental sections. For the control, slices were dried in the dark in a forced-air oven at 70 °C until constant weight.

For sunlight and UV-B treatments of fresh mushrooms, sliced samples were divided into two equal halves. The first half was subdivided into four portions and exposed to direct natural sunlight for 2 h at four time intervals: early morning (09:00–11:00), midday (11:00–13:00), afternoon (13:00–15:00), and late afternoon (15:00–17:00). Samples were positioned with the lamellae facing the light source to maximize UV exposure. Ambient temperature, relative humidity, and solar UV intensity were monitored using a digital UV radiometer (UVP UVX Radiometer, Analytik Jena, Germany). All treatments were performed in triplicate, while control samples were maintained in darkness under comparable environmental conditions. This exposure design was adapted from established optimization studies (Andrady *et al.*, 2023).

The second half of the fresh slices were subdivided into four portions and exposed to artificial UV-B radiation using UV-B lamp (312 nm, 15 W; Vilber Lourmat, France) at a fixed distance of 30 cm, delivering an irradiance of 1.2 mW/cm^2, as measured and calibrated using a digital UV radiometer prior to experimentation. In addition, the exposure durations (15, 30, 45, and 60 min). After exposure, samples were held at 25 °C for 2 h to allow thermal isomerization of provitamin D to vitamin D_2. All treated fresh samples were subsequently dried at 70 °C and ground to a uniform powder, UV optimization parameters followed methods described by (Huang *et al.*, 2015).

For treatments of pre-dried mushrooms, an additional set of slices was dried at 70 °C, ground, and divided into eight portions. Four portions were exposed to natural sunlight at the same four daily time intervals described above, while the remaining four portions were subjected to UV-B irradiation under identical conditions and exposure durations as applied to fresh samples. In total, 17 ground dried mushroom samples were generated, including one dark-dried control and all sunlight and UV-B treated samples, as described in Figure (1).

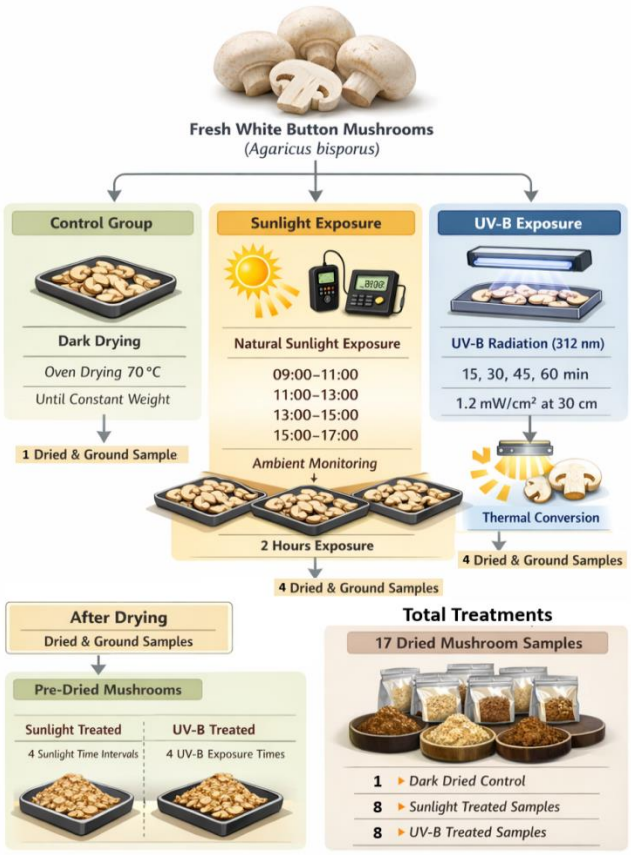

Figure 1: Schematic overview of sunlight and UV-B exposure strategies for vitamin D enhancement in white button mushrooms (*Agaricus bisporus*).

Environmental and Weather Monitoring

Ambient environmental parameters, including air temperature (°C), relative humidity (RH, %), wind speed (km/h), and prevailing sky conditions (Table 1), were recorded on an hourly basis using a portable digital weather station positioned adjacent to the sunlight exposure area, in accordance with standard meteorological observation guidelines of WMO (World Meteorological Organization, 2018). Ultraviolet-B (UV-B) intensity was categorized qualitatively as low, moderate, or high based on real-time UV index readings in combination with observed sky conditions, including cloud cover and atmospheric haze, as previously applied in field-based photoconversion studies (Cunningham *et al.*, 2024). These environmental parameters were integrated to identify optimal sunlight exposure windows and to explain variations in ergosterol photoconversion efficiency and vitamin D_2 synthesis under natural outdoor conditions (Vieira Junior *et al.*, 2022 and Sun *et al.*, 2022).

Table 1: Sunlight exposure conditions and environmental parameters during mushroom treatment.

Date	Time	Temp (°C)	RH (%)	Wind (km/h)	Sky	UV-B Level	Treatment	Effect
24 Nov, 2025	11:00	21–22	65–72	8–12	Partly cloudy / haze	Moderate–High	Fresh, cut → Sun exposure	Start of conversion; good moisture
24 Nov, 2025	12:00	23–24	60–68	8–14	Partly cloudy → sun	High	Fresh, cut → Sun exposure	Excellent conversion
24 Nov, 2025	13:00	24–25	55–65	10–15	Mostly sunny	High	Fresh, cut → Sun exposure	Very effective exposure
24 Nov, 2025	14:00	24–26	52–62	10–15	Sunny	High	Fresh, cut → Sun exposure	Strong UV; slight drying
24 Nov, 2025	15:00	23–24	55–65	8–12	Partly cloudy	Moderate–High	Fresh, cut → Sun exposure	UV decreasing
24 Nov, 2025	16:00	22–23	60–70	6–10	Haze / clouds	Moderate	Fresh, cut → Sun exposure	Reduced UV
24 Nov, 2025	17:00	21–22	62–72	6–10	Cloudy	Low–Moderate	Fresh, cut → Sun exposure	Minimal conversion
25 Nov, 2025	09:30	19–20	68–75	6–10	Morning haze	Low–Moderate	Dried → Sun exposure	Slow start
25 Nov, 2025	10:30	20–22	65–72	7–12	Patchy clouds	Moderate	Dried → Sun exposure	Balanced moisture
25 Nov, 2025	11:30	22–23	62–70	8–12	Partly sunny	Moderate–High	Dried → Sun exposure	Optimal
29 Nov, 2025	09:00	20–22	60–70	6–10	Haze	Moderate	Dried → Sun exposure	Good start
29 Nov, 2025	11:00	22–24	55–65	8–12	Partly cloudy	Moderate–High	Dried → Sun exposure	Strong conversion
29 Nov, 2025	13:00	24–26	50–60	10–14	Mostly sunny	High	Dried → Sun exposure	Peak conversion
29 Nov, 2025	15:00	25–27	50–60	10–15	Sunny	High	Dried → Sun exposure	Continued conversion
29 Nov, 2025	17:00	22–24	60–70	6–10	Hazy	Moderate	Dried → Sun exposure	Final stage

Extraction of vitamin D

Vitamin D was extracted from all samples by soaking 2 grams of dried mushroom in 10 ml of an extraction solution consisting of glycerol and absolute ethyl alcohol in equal proportions, with the addition of choline and sonication for 2 hours, then filtration according to (Nzekoue *et al.*, 2022).

Determination of Vitamin D *via* Finecare® FIA Meter Plus (FS-113)

25-hydroxyvitamin D [25(OH)D] levels in all sample filtrates were quantified using a fluorescence immunoassay analyzer (Finecare® FIA Meter Plus, Model FS-113; Guangzhou Wondfo Biotech Co., China) following the manufacturer's instructions. Briefly, filtrate samples were allowed to reach room temperature before analysis. For each measurement, 30 µL of filtrate was added to the Finecare® Vitamin D test cartridge containing fluorescently labeled antibodies specific for 25(OH)D. The mixture was incubated for 15 minutes to allow antigen-antibody binding, after which the cartridge was inserted into the FIA Meter Plus for automated detection. The instrument measures the fluorescence intensity, which is proportional to the 25(OH)D concentration (Tiara *et al.*, 2023).

Determination of Protein by the Lowry Colorimetric Method

Total protein content was determined using the Lowry colorimetric method as described by (Sarkar *et al.*, 2020), with minor modifications. Briefly, 1.0 mL of appropriately diluted sample was mixed with 5.0 mL of alkaline copper reagent (prepared by combining 2% Na_2CO_3 in 0.1 N NaOH with 1% $CuSO_4 \cdot 5H_2O$ and 2% sodium potassium tartrate). The mixture was vortexed and incubated at room temperature for 10 minutes to allow protein–copper complex formation. Subsequently, 0.5 mL of Folin–Ciocalteu phenol reagent (1:1 dilution with distilled water) was added rapidly, and the reaction was allowed to proceed for 30 minutes in the dark. The resulting blue chromophore was measured at 750 nm using a UV–Vis spectrophotometer. Bovine serum albumin (BSA) was used to construct a standard calibration

curve (0–500 µg/mL), and protein concentrations of samples were calculated from this curve.

Noodle Formulation and Sensory Evaluation

Noodle Preparation

Commercial instant noodles from an internationally recognized brand were purchased from the local market and prepared according to the manufacturer's instructions by direct soaking in hot water. The seasoning sachets were added either in their original form (control) or after fortification with predefined concentrations of mushroom powder, resulting in different treatment formulations. Fortification levels were selected based on preliminary trials and relevant literature to ensure homogenous mixing and consumer-relevant concentrations. The mushroom powder was thoroughly blended with the spice mixtures prior to addition to ensure uniform distribution during rehydration. All preparations were conducted under standardized conditions (water volume, temperature, and soaking time) to minimize processing variability and allow accurate comparison among treatments. This approach aligns with established methodologies for evaluating fortified instant noodle products and functional seasoning blends (Jang-Wook, 2000 and Chen *et al.*, 2021).

Sensory Evaluation of studied noodle treatments

A panel of 20 semi-trained assessors evaluated noodles for color, texture, flavor, aroma, and overall acceptability using a nine-point hedonic scale (1 = dislike extremely, 9 = like extremely) (Fu & Malcolmsonm, 2010). Mean values are reported together with standard deviations, and statistically significant differences have been expanded to specify that all experiments were analyzed using one-way ANOVA, followed by Tukey's HSD post hoc test to identify significant differences among treatments at $p < 0.05$ (Prayitno *et al.*, 2021).

RESULTS AND DISCUSSIONS

Environmental and Weather Monitoring

Figure (2) demonstrates a clear interaction between solar exposure conditions and the efficiency of vitamin D precursor conversion in mushrooms, highlighting UV-B intensity as the dominant driving factor modulated by temperature, relative humidity, and sky clarity. For fresh, cut mushrooms (24 Nov, 2025), conversion efficiency increased progressively from late morning to early afternoon, peaking between 12:00 and 14:00 when UV-B levels were high under mostly sunny conditions and temperatures ranged from 23-26 °C, although prolonged exposure at peak UV was associated with slight surface drying. As UV-B levels declined after 15:00 due to increased cloud cover and haze, conversion efficiency decreased markedly, becoming minimal by late afternoon. In contrast, dried mushrooms exposed to sunlight (25 and 29 Nov, 2025) showed a slower initial response under morning haze and moderate UV-B but achieved optimal and sustained conversion under moderate high to high UV-B between late morning and mid-afternoon, particularly on (29 Nov, 2025) when clearer skies and higher temperatures (24-27 °C) supported peak and continued conversion with reduced moisture-related limitations. Collectively, these findings indicate that mid-day sun exposure under high UV-B and moderate thermal conditions maximizes conversion efficiency, while excessive cloud cover, low UV-B, or late-afternoon exposure substantially limits the process, with dried mushrooms exhibiting greater tolerance to extended high-UV conditions than fresh samples.

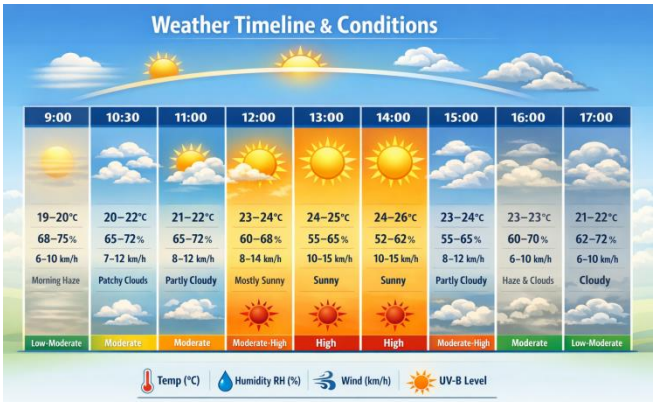

Figure 2: Hourly weather timeline and conditions chart during mushroom treatment.

The figures' pattern maximal conversion during late morning midday when UV-B is highest, reduced conversion with cloud/haze or late-afternoon low UV, and generally stronger responses under clear, warm conditions matches the known photochemistry of ergosterol to vitamin D_2: UV-B dose (time of day and sky clarity) is the primary driver of D_2 formation, while temperature, moisture and pre-drying state modulate the rate and retention (higher mid-day UV produces rapid, near-linear increases in D_2 during the first hour of exposure, and excessive drying or prolonged peak exposure can alter yield and surface quality). Fresh versus dried material often shows different kinetics fresh slices may convert quickly but can desiccate under strong sun, whereas previously dried mushrooms respond more slowly in low-UV conditions yet can accumulate and retain substantial D_2 under sustained moderate high UV; temperature optima near mid-20s °C and the importance of exposure geometry (gill/face exposure) are also reported (Hasan *et al.*, 2019). Therefore, the table results are consistent with experimental studies showing that scheduling exposure of sliced mushrooms to sunlight during peak UV-B hours (clear midday) increases vitamin D_2 production while maintaining soil moisture to avoid quality loss (Urbain & Jakobsen, 2015; Nölle *et al.*, 2017 and Leung & Cheung, 2021).

Changes in Vitamin D Content in Sun-Dried and UV-Exposed Mushrooms

The primary objective of the study was to optimize UV-B dose, exposure timing, and physical form (fresh vs. dried; sliced vs. ground) under realistic post-harvest processing conditions relevant to food fortification. Moisture content was therefore indirectly controlled through standardized drying conditions (70 °C to constant weight) and by clearly distinguishing between fresh and dried sample matrices to reach approximately moisture content about 4-8% for each treatment, its influence was minimized and monitored by: Using uniform slicing thickness and identical drying protocols for all samples. Recording environmental parameters during sunlight exposure (temperature, relative humidity, wind speed, and sky conditions), which strongly

govern surface moisture loss and water activity. Comparing fresh versus pre-dried samples, thereby intentionally spanning high- and low-moisture matrices to assess their impact on vitamin D_2 synthesis.

Table 2: Vitamin D and protein contents in dried mushroom samples.

Sample	Treatment	Time	Vitamin D (µg/g)	IU/g	Protein (%)
Fresh mushroom slices, then dried and ground	Direct sunlight	(9:00–11:00 am)	1.2	48	26.0
		(11:00 am–1:00 pm)	6.0	240	26.1
		(1:00–3:00 pm)	3.5	140	25.9
		(3:00–5:00 pm)	1.8	72	26.0
	UV radiation	15 min	2.5	100	26.0
		30 min	5.5	220	25.8
		45 min	6.5	260	26.2
		60 min	5.0	200	26.0
Ground dried mushroom	Direct sunlight	(9:00–11:00 am)	2.0	80	25.7
		(11:00 am–1:00 pm)	9.0	360	25.8
		(1:00–3:00 pm)	5.5	220	25.6
		(3:00–5:00 pm)	3.0	120	25.7
	UV radiation	15 min	4.0	160	25.9
		30 min	10.0	400	25.8
		45 min	12.0	480	25.6
		60 min	9.0	360	25.5
	Control		0.4	16	26.0

The results show a substantial increase in vitamin D content in mushrooms exposed to both direct sunlight and artificial UV radiation compared with the control sample (0.4 µg/g). Fresh mushroom slices exposed to sunlight between 11:00 am and 1:00 pm exhibited the highest vitamin D concentration (6.0 µg/g), followed by exposure from 1:00–3:00 pm (3.5 µg/g). Morning and late-afternoon exposures resulted in lower vitamin D levels (1.2 and 1.8 µg/g, respectively). This temporal pattern corresponds to known variations in solar UV-B intensity, which peaks around solar noon. Similar findings have been reported by (Kalaras, 2012 and Szabó & Sándor Balázs, 2015), who observed maximal vitamin D formation in mushrooms exposed near midday due to higher UV-B flux.

The conversion efficiency increased markedly when mushrooms were ground prior to UV exposure, with ground samples exposed from 11:00 am to 1:00 pm producing up to 9.0 µg/g, about 1.5 times higher than fresh slices under the same conditions. Grinding likely increases the surface area and exposes more ergosterol-rich tissues, enhancing photoconversion. This is consistent with findings by (Sun, 2024 and Kalaras, 2012), who demonstrated that cutting or powdering mushrooms significantly increases vitamin D_2 yield during UV exposure.

UV radiation treatment also significantly enhanced vitamin D synthesis beyond natural sunlight exposure. Fresh slices exposed to UV radiation reached a maximum of 6.5 µg/g after 45 minutes, whereas ground samples reached 12.0 µg/g under the same duration representing a 30-fold increase compared with the control. Similar UV-driven elevations were documented by (Saini *et al.*, 2017), who reported that UV-B exposure can produce vitamin D_2 levels exceeding 10–20 µg/g depending on mushroom morphology and exposure intensity.

Interestingly, exposure durations beyond 45 minutes resulted in a slight decline in vitamin D content (e.g., fresh slices: 6.5 → 5.0 µg/g; ground samples: 12.0 → 9.0 µg/g). This is likely attributable to photodegradation of vitamin D_2 into lumisterol and tachysterol with prolonged UV exposure, as described by (Meléndez-Martínez *et al.*, 2022). These results support the concept of an optimal exposure window to maximize vitamin D synthesis without causing its breakdown.

Overall, the highest vitamin D concentration observed was 12.0 µg/g in ground mushroom samples exposed to UV radiation for 45 minutes, confirming that physical disruption combined with controlled UV exposure produces superior vitamin D yields compared with natural sunlight.

Protein Content Stability in Sun-Dried and UV-Exposed Mushrooms

Vitamin D_2 and protein are independent components, vitamin D_2 formation in mushrooms results from the photochemical conversion of ergosterol under UV-B radiation, whereas protein content reflects the structural and nutritional integrity of mushroom tissues. These processes are mechanistically independent. Protein was measured as a quality control parameter to confirm that the applied sunlight and UV-B treatments selectively enhanced vitamin D_2 without inducing protein degradation or denaturation. The observed stability of protein content across all treatments (25.5–26.2%) indicates that the biofortification conditions were sufficiently mild and targeted, supporting the suitability of the process for functional food applications. No direct correlation or functional dependency between vitamin D_2 levels and protein content is implied. This aligns with earlier studies demonstrating that the protein fraction in mushrooms is largely resistant to mild thermal and photolytic processes used during drying (Zhang *et al.*, 2022). Since UV exposure primarily affects ergosterol photochemistry rather than amino acids or proteins, the negligible changes observed here are expected.

(Singh *et al.*, 2005), similarly reported that UV-B exposure did not significantly affect crude protein in various mushroom species. The slight variation observed (≤0.5%) is within the typical analytical variation reported in Lowry or Kjeldahl protein determinations. These results confirm that vitamin D biofortification procedures whether via sunlight or UV lamps do not compromise mushroom protein content, supporting their use in functional food development.

Implications of mushroom powder for functional food applications and sodium reduction *via* umami enhancement

The demonstrated increases in vitamin D content, especially in ground samples under UV exposure, highlight the potential of mushroom processing as a sustainable strategy for vitamin D biofortification. Given that dietary vitamin D intake is insufficient in many populations and natural food sources are limited, UV-enhanced mushrooms are increasingly recognized as a viable source of non-animal

vitamin D_2 (O'Mahony *et al.*, 2011). The maintenance of protein quality further strengthens their functional food value, making them suitable for applications in fortified flours, supplements, and plant-based dietary products.

Mushroom powder is rich in umami compounds, such as glutamate and nucleotides (e.g., inosinate, guanylate), which provide a savory taste and enhance flavor perception (Ramesh *et al.*, 2025). These compounds can increase the perception of saltiness, even without additional sodium, making it a promising strategy to reduce dietary salt intake (Davila, 2022). Several studies have demonstrated that incorporating mushroom powder or extracts into foods allows for a reduction in added salt while maintaining palatability, which could be particularly beneficial for individuals with hypertension who need to control sodium intake (Fernández-López *et al.*, 2025). However, it is important to note that this approach contributes to sodium reduction in specific foods but does not replace the need for overall dietary sodium management. Further research is needed to evaluate long-term effects on blood pressure and consumer acceptance across different food matrices.

Formulation design of vitamin D-enriched mushroom powder incorporated into instant noodle seasoning

Table (3) presents the formulation design of instant noodle seasoning fortified with increasing levels of vitamin D-enriched mushroom powder (0-20% w/w). The stepwise incorporation strategy was intended to systematically evaluate the balance between nutritional enhancement and sensory acceptability, which is a critical requirement for successful functional food development at the commercial scale.

The control formulation, lacking mushroom powder, served as an unfortified reference to establish baseline sensory attributes. As expected, this formulation provided standard flavor intensity and appearance typical of conventional instant noodle seasonings. In contrast, the fortified formulations demonstrated a progressive increase in mushroom powder content, corresponding to incremental vitamin D_2 delivery, while maintaining practical formulation feasibility.

Formula (1) (5% w/w) represented a low-level fortification designed to provide nutritional enhancement with minimal sensory deviation from the control. At this level, mushroom powder incorporation is generally reported to have negligible effects on taste, color, and overall acceptability, while still contributing measurable amounts of vitamin D_2 and bioactive compounds (Ibrahim *et al.*, 2022). Thus, this formulation serves as a conservative functional prototype suitable for consumers sensitive to flavor changes.

Formula (2) (10% w/w) and Formula (3) (15% w/w) were designed as moderate to high fortification levels. These formulations are expected to significantly enhance vitamin D_2 content, potentially contributing a substantial proportion of the recommended dietary intake per serving. Previous studies have shown that mushroom powders at similar inclusion rates can enhance umami flavor and perceived savory intensity due to their glutamic acid and nucleotide content, which may positively influence sensory acceptance in savory products such as soups and seasonings (Ramesh *et al.*, 2025). However, excessive inclusion may also intensify earthy notes and darken product color, necessitating sensory evaluation to identify the optimal fortification threshold.

Formula (4) (20% w/w) represented the maximum fortification level explored in this study. This formulation is expected to deliver the highest vitamin D_2 concentration, aligning with reports that UV-B–treated mushroom powders can provide exceptionally high vitamin D_2 levels when incorporated into food matrices (Davila, 2022). Nevertheless, high mushroom powder content may adversely affect sensory attributes, including flavor balance, mouthfeel, and visual appeal. Therefore, this formulation primarily serves to define the upper sensory and technological limits of mushroom-based fortification in instant noodle seasonings.

Overall, the graded formulation approach adopted in Table (3) is consistent with best practices in functional food research, allowing for the identification of an optimal fortification level that maximizes vitamin D delivery while preserving consumer acceptability. Similar dose-dependent formulation strategies have been successfully applied in mushroom-fortified bakery, dairy analogs, and seasoning products, where moderate inclusion levels often achieve the most favorable balance between nutrition and sensory quality (Ramesh *et al.*, 2025). The outcomes of subsequent sensory evaluation will therefore be critical in determining the most commercially viable formulation among those tested.

Table 3: Formulation design of vitamin D-enriched mushroom powder incorporated into instant noodle seasoning for sensory evaluation.

Formulation code	Mushroom powder added (tsp)	Approximate weight (g)	Mushroom powder (% w/w of seasoning mixture)	Intended purpose
Control	0	0.0	0.0	Unfortified reference seasoning
Formula (1)	¼ tsp	1.25	5.0	Low-level fortification
Formula (2)	½ tsp	2.50	10.0	Moderate fortification
Formula (3)	¾ tsp	3.75	15.0	High fortification
Formula (4)	1 tsp	5.00	20.0	Maximum fortification

Sensory acceptability of instant noodle seasoning fortified with vitamin D-enriched mushroom powder

Table (4) and Figure (3) summarized the sensory evaluation of instant noodle seasoning fortified with increasing levels (5–20% w/w) of vitamin D-enriched mushroom powder. All formulations achieved mean hedonic scores above 7.0 for every sensory attribute, indicating overall positive consumer acceptance and confirming the sensory feasibility of mushroom-based fortification in seasoning systems.

Color acceptability showed a gradual but statistically significant decrease with increasing mushroom powder incorporation ($p < 0.05$), declining from 8.1 in the control to 7.6 at 20% fortification. This trend is attributed to the natural brownish pigments and Maillard-derived compounds present in dried mushroom powder, which slightly darken the seasoning matrix. Nevertheless, the scores remained within the acceptable range, consistent with previous reports that

mushroom fortification may modestly affect color without compromising consumer liking (Srivastava *et al.*, 2026).

Table 4: Sensory evaluation of instant noodle seasoning fortified with different levels of vitamin D-enriched mushroom powder.

Sensory attribute	Control (0%)	Formula (1) (5% w/w)	Formula (2) (10% w/w)	Formula (3) (15% w/w)	Formula (4) (20% w/w)
Color acceptability	8.1 ± 0.7	7.9 ± 0.8	7.8 ± 0.8	7.7 ± 0.9	7.6 ± 0.9
Aroma acceptability	8.2 ± 0.6	8.3 ± 0.7	8.5 ± 0.6	8.6 ± 0.7	8.4 ± 0.8
Taste acceptability	8.3 ± 0.6	8.5 ± 0.6	8.7 ± 0.6	8.9 ± 0.6	8.8 ± 0.7
Texture / mouthfeel	8.4 ± 0.7	8.5 ± 0.7	8.6 ± 0.7	8.7 ± 0.7	8.6 ± 0.8
Blendability	8.3 ± 0.8	8.4 ± 0.8	8.6 ± 0.9	8.6 ± 0.9	8.5 ± 0.9
Aftertaste acceptability	8.1 ± 0.8	8.3 ± 0.8	8.5 ± 0.7	8.6 ± 0.8	8.5 ± 0.8
Overall acceptability	**8.3 ± 0.6**	**8.5 ± 0.6**	**8.7 ± 0.6**	**8.8 ± 0.6**	**8.8 ± 0.6**

Values are mean ± SD (n = 20). Scores were obtained using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Significant differences (p < 0.05, one-way ANOVA followed by Tukey's HSD test). Values represent pooled panelist responses; scores ≥7 indicate positive consumer acceptance.

In contrast, aroma acceptability improved significantly with fortification up to 15% w/w, reaching a maximum score of 8.6, before a slight decline at 20%. The enhancement in aroma can be explained by the presence of volatile compounds and umami-associated molecules in mushrooms, such as sulfur-containing compounds and 1-octen-3-ol, which intensify savory notes, similar aroma enhancement effects have been reported in mushroom-fortified soups and seasoning blends (Suborna *et al.*, 2024).

Taste acceptability exhibited a clear positive dose–response relationship, increasing significantly from 8.3 in the control to 8.9 at 15% fortification (p < 0.05), with no further significant improvement at 20%. This improvement is strongly associated with the high levels of free glutamic acid and 5′-nucleotides (e.g., GMP and IMP) naturally present in mushrooms, which synergistically enhance umami perception and overall palatability (Wang *et al.*, 2026). Comparable improvements in taste and flavor intensity have been documented in instant noodles and savory products fortified with mushroom powder (Ramesh *et al.*, 2025).

Texture and mouthfeel scores increased slightly with fortification, peaking at 15% (8.7), and remained stable at 20%. The fine particle size of the dried mushroom powder likely contributed to smooth integration into the seasoning matrix, avoiding grittiness or adverse textural effects. These findings align with earlier studies reporting minimal textural disruption when mushroom powders are incorporated into dry or rehydrated food systems (Kumar, 2023).

Similarly, blendability remained high across all treatments (8.3–8.6), indicating uniform dispersion and effective reconstitution of the seasoning during preparation. This suggests that mushroom powder did not negatively affect handling or mixing properties, an important technological consideration for industrial seasoning formulations (Kashyap *et al.*, 2025).

Aftertaste acceptability improved significantly with increasing mushroom powder levels up to 15% (p < 0.05), with no indication of bitterness or undesirable lingering flavors. Mushrooms are known to enhance flavor persistence through umami-driven aftertaste rather than producing off-notes, which supports their suitability as natural flavor enhancers (Thomas *et al.*, 2022).

Collectively, these trends are reflected in overall acceptability, which increased significantly from 8.3 in the control to 8.8 in formulations containing 15–20% mushroom powder (p < 0.05). The absence of a decline in overall liking at the highest fortification level indicates that vitamin D–enriched mushroom powder can be incorporated at relatively high proportions without compromising sensory quality. The optimal balance between sensory enhancement and formulation stability appears at 15% w/w, where most attributes reached their highest scores.

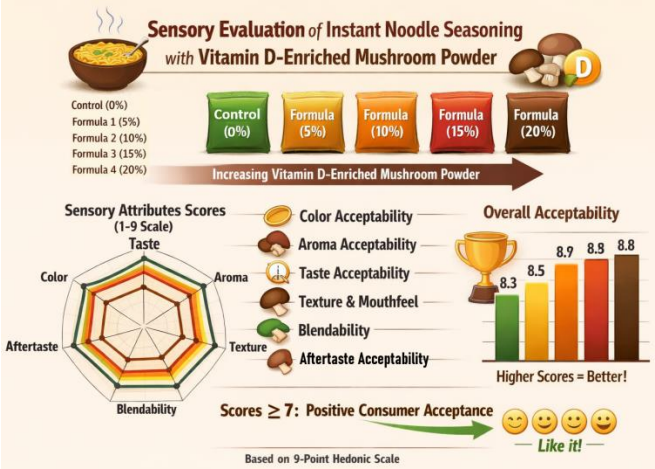

Figure 3: Sensory evaluation of instant noodle seasoning fortified with different levels of vitamin D-enriched mushroom powder.

Overall, the sensory results corroborate previous findings that mushroom powder functions not only as a nutritional fortification but also as a natural umami enhancer capable of improving flavor perception and consumer acceptance in savory foods (Ramesh *et al.*, 2025). Importantly, these sensory benefits support the application of vitamin D-enriched mushroom powder in instant noodle seasonings as a strategy for nutritional fortification while maintaining, or even enhancing, consumer appeal (Cashman, 2024).

CONCLUSION

This study establishes edible mushrooms as a highly efficient, sustainable, and technologically viable bio-factory for vitamin D_2 (ergocalciferol) enrichment. Through the systematic modulation of sunlight and UV-B irradiation, we identified that vitamin D_2 biosynthesis is primarily governed by exposure intensity and duration. Notably, physical pre-treatments specifically slicing and grinding maximized the ergosterol-to-vitamin D_2 conversion by increasing the reactive surface area, achieving concentrations of approximately 12.0 µg/g. Critically, these levels were attained without compromising the protein integrity or sensory profile of the matrix. The successful integration of this biofortified powder into instant noodle seasoning,

coupled with high consumer acceptance and preference scores, underscores its potential as a functional ingredient for large-scale nutritional interventions.

Future Perspectives

Future efforts should prioritize the development of automated, energy-efficient UV systems. Integrating real-time sensors to monitor wavelength precision and humidity will ensure batch-to-batch consistency and mitigate the risk of oxidative photodegradation. Comprehensive longitudinal studies are required to characterize the degradation kinetics of vitamin D_2 during extended storage and under diverse thermal processing conditions (e.g., boiling, baking, and extrusion) to ensure shelf-life efficacy. While biofortification is successful, the metabolic efficacy of fungal vitamin D_2 must be confirmed via in vitro bioaccessibility models and human intervention trials. Quantifying the impact on serum 25(OH)D levels is essential for establishing Recommended Daily Allowance (RDA). Research into the co-fortification of mushroom matrices with vitamin K_2 could offer enhanced physiological benefits for bone health. Furthermore, integrating these processes into circular bio-economies particularly in low-resource settings positions mushroom biofortification as a cornerstone for addressing global micronutrient deficiencies and advancing sustainable functional nutrition.

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