ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

VITAMIN D2 ENHANCEMENT OF PRE-TREATED BUTTON MUSHROOM BY NATURAL UV RADIATIONS AND ITS EFFECT ON NUTRIENT CONTENT AND ANTIOXIDANT PROPERTIES

SINGH S* AND JOSHI I

Department of Home Science, IIS (deemed to be University), Jaipur, India, 302020

***Corresponding Author: Simran Singh: E Mail: simran.singh@iisuniv.ac.in**

Received 16th Jan. 2023; Revised 7th April 2023; Accepted 3rd July 2023; Available online 1st Feb. 2024

https://doi.org/10.31032/IJBPAS/2024/13.2.7806

ABSTRACT

Vitamin D deficiency is a global problem and is an underlying cause of several chronic diseases. Mushrooms are the reservoirs of many essential nutrients and bio-active compounds. Upon exposure to sunlight or UV radiations, mushrooms can synthesize vitamin D2 due to the presence of its precursor, ergosterol. Inclusion of vitamin D2 enhanced mushrooms in the diet can be a highly cost-effective method to improve the vitamin D consumption globally. As mushrooms are highly perishable and undergo browning, various pre-treatments like washing and soaking in potassium metabisulphite, citric acid, vinegar, salt and sugar solutions are applied to reduce enzymatic browning, microbial load and increase shelf life. This study aims to compare the changes occurring in vitamin D2 content, nutritive value and antioxidant activity in button mushrooms exposed to natural UV radiation from sunlight (T-1) with those occurring in button mushrooms dipped in potassium metabisulphite prior to exposure to sunlight (T-2) and assess the stability of vitamin D2 over a period of three months. All the samples were analyzed using standard methods and compared against control. Vitamin D2 content in the T-1 sample was significantly ($p \leq 0.05$) higher than T-2 and control. Significant effect ($p \leq 0.05$) of treatments was also noted in the moisture, ash, fibre and fat content of samples. Non-significant differences were seen in the protein, carbohydrate, calcium and iron content of the samples. Powder from both the treatments retained higher antioxidant properties. A gradual fall in the vitamin D2 content was noted in all samples stored at ambient temperature over the period of three months.

Keywords: ***Agaricus bisporus,*** **Mushroom, Potassium metabisulphite, Vitamin D, UV**

INTRODUCTION

Vitamin D is a vital micronutrient playing a valuable role in absorption of calcium and promoting bone health **[1]**. It also plays an important role in inflammation, immune function, cell differentiation and proliferation **[2]**. Insufficiency of vitamin D can cause problems like rickets, osteomalacia, muscle pain, reduced bone mineral density and a higher incidence of falls and fractures **[3]**. Studies also suggest that subclinical, asymptomatic vitamin D deficiency is also associated with a number of non-skeletal diseases such as cancer, diabetes, hypertension, cardiovascular disease, stroke, multiple sclerosis, inflammatory bowel disease, mental illness, liver diseases and autoimmune diseases **[4, 5]**. It has been observed that people with higher serum 25-hydroxy-D levels had lower COVID-19 mortality rates **[6]**. Roughly 1 billion people all over the world are vitamin D deficient and nearly half of the global population has vitamin D insufficiency **[7]**. As vitamin D deficiency is a global problem, offering a rich, natural and economical source of this vitamin in the family's food basket is significant for improving community health and reducing morbidity and death rates **[8]**.

Vitamin D (calciferol), is a fat-soluble vitamin with two major forms - vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) which differ in their side chains **[9]**. Ultraviolet B (UVB) radiation of skin exposed to sunlight is the primary source of Vitamin D3. However, as a result of lifestyle modifications, frequent sun exposure has diminished, leading to a vitamin D deficit **[10]**. Further, at higher latitudes, synthesis of vitamin D through skin is minimal in the winter months **[11]**. Important dietary sources of vitamin D3 are egg yolk, fatty fish, beef liver, and fortified dairy products which are of animal origin and may not be suitable for vegetarian and vegan populations **[12]**. Vitamin D2 is found in fungi and yeast. Most wild mushrooms are an excellent non-animal source of vitamin D2 **[13]**. Cultivated mushrooms, on the other hand, have a little preformed vitamin D as they are grown in dark chambers. However, the vitamin D2 content of mushrooms can be enhanced post-harvest upon exposure to ultraviolet radiations. A high concentration of ergosterol (provitamin D2) is present in the cell walls of mushrooms which upon exposure to UV rays from sunlight or artificial sources gets converted into previtamin D2 and later on to Vitamin D2. The nature of these reactions is similar to those occurring in skin **[14]**. Hence, Vitamin D enhanced mushrooms can be a valuable alternative for vegetarian populations.

Further, mushrooms also contain many other essential nutrients and bioactive compounds with numerous therapeutic and health benefits **[15]**. The functional nutrients in mushrooms are proteins, amino acids, dietary fibre, polyunsaturated fatty acids (PUFA), terpenoids, steroids, keto acids, vitamins, minerals and antioxidants like glutathione and selenium **[16]**. Commonly cultivated mushrooms worldwide are *Agaricus bisporus* (button mushroom), *Lentinus edodes* (shiitake mushroom), *Pleurotus* spp. (oyster mushroom), and *Flammulina velutipes* (enoki mushroom) with button mushrooms dominating the global commercial mushroom industry **[17]**.

Mushrooms are mainly marketed in fresh form but they are highly perishable and hence special postharvest treatments are needed to protect them **[18]**. Their market value falls within two-three days after harvest due to water loss, senescence, browning and microbial attack **[19]**. Mushrooms are susceptible to quick enzymatic browning due to their high tyrosinase and phenolic contents **[20]**. Washing mushrooms with anti-browning and anti-microbial agents have lately received commercial acceptance as a means to remove casing soil particles and for the application of microbial and browning inhibitors **[21, 22]**. The most common pre-treatments are washing and soaking in potassium metabisulphite (KMS), citric acid, sugar and salt alone or in combination **[23]**. Washing in potassium metabisulphite (KMS) significantly improves the whiteness of button mushrooms with a lesser rate of browning during storage at ambient temperature and refrigerated conditions **[24]**.

A number of studies have been conducted in the past, focussing on vitamin D2 enrichment in popular mushroom varieties across the world. The various techniques studied for vitamin D2 enhancement include sun exposure, sun drying, and different types of UV radiations like UVA, UVB, UVC and Pulsed UV radiations. Exposure of mushrooms to sunlight even for a short duration can result in nutritionally significant increase in vitamin D2 **[25]** and is the most practical, simple and inexpensive consumer-based approach to increase the vitamin D intake of populations.

However, to the best of the authors' knowledge, no study has been conducted so far to study the effect of pre-treatment of mushrooms like dipping in KMS solution on the enhancement of vitamin D2 content upon sunlight exposure. Hence, the present study had been planned to compare the changes occurring in vitamin D2 content, nutritive value and antioxidant activity in button mushrooms exposed to natural UV from sunlight with those occurring in mushrooms dipped in KMS prior to exposure to sunlight. Another purpose of the study was to assess the stability of vitamin D2 thus enhanced, in mushroom powder stored at conditions similar to those used by general consumers.

MATERIALS AND METHODS

Procurement of Mushrooms

Freshly harvested, blemish-free, unwashed Button mushrooms (*Agaricus bisporus* strain NCS-100) from the same flush were procured directly from the supplier. Mushrooms were brought from the facility to the lab before sunrise in opaque containers, where they were stored at 4˚C and processed the same day. The average hat diameter (n=10) of mushrooms was 37.4±2.79 mm. All the mushrooms were cleaned, washed with distilled water and allowed to drain on a clean cheesecloth. The mushrooms were then divided into three batches of one kg each for different treatments.

Application of treatments

Enhancement of vitamin D2 in button mushrooms was carried out using two processing techniques. For Treatment-1(T-1), mushrooms were sliced and exposed to natural UV radiation from sunlight for three hours. For Treatment -2 (T-2), mushrooms were dipped in 1 per cent KMS solution for 15 minutes, followed by slicing and then exposed to sunlight for three hours. The control group (C) was obtained by subjecting mushrooms to the same slicing, drying and grinding method without natural UV exposure from sunlight. For all the three batches, mushrooms were sliced vertically into nearly 3.0 mm thickness. Sun exposure of sliced mushrooms (T-1 & T-2) was carried out in mid-February between 11.00 am to 3.00 pm on a sunny day at IIS University, Jaipur (26.84˚N, 75.77˚E, 431m above sea level). The procedure followed for vitamin D2 enhancement of mushrooms using sun exposure was based upon techniques used in secondary researches **[26, 27]**. Mushroom slices were spread in single-layer on food-grade LDPE (low-density polyethylene) sheets and irradiated on both sides. The sides were turned after one and a half hours to get the maximum exposure benefit. On the day of experiment, the environmental temperature during exposure ranged between 26- 28˚C, the UV index between 6-7 and the relative humidity was 24 per cent. To avoid further exposure to UV light, sun-exposed mushroom slices were carefully placed back into opaque containers and taken for drying. Respective mushroom slices along with the control batch were spread in a single layer in trays and subjected to drying in a hot air oven at 60˚C for 8-10 hours. The dried mushroom slices were pulverized to a fine powder in an electric grinder and packed in aluminium coated polyethylene bags and stored at ambient temperature in darkness in tightly closed plastic containers for 3 months for further analysis.

Vitamin D2 analysis of button mushroom powder

Immediately following the powder preparation and after 30, 60 and 90 days of storage, vitamin D2 was extracted and analysed by high performance liquid chromatography (HPLC) using the method described by Rathore *et al*., **[28]**, albeit with slight modifications at the Quality Control Laboratory at Amol Pharmaceuticals, Jaipur. Button mushroom powder (2g) was weighed accurately and four ml of sodium ascorbate solution (Sigma chemicals), 50 ml of 95 per cent pure ethanol (Merck, Germany) and 10 ml of 50 per cent potassium hydroxide solution (85% pure, Merck Chemicals) were added. Saponification of the mixture was carried under reflux for one hour at 80°C, followed by immediate cooling to room temperature and transfer to a separating funnel. Extraction of the mixture was carried out first with 15 ml each of de-ionized water and ethanol followed by the addition of 50, 50 and 20 ml of n-hexane in three stages. The organic layers were pooled and three times washed with 50ml of ethanolic potassium hydroxide (3%) and then with de-ionized water to a neutral pH. The organic layer was rotary evaporated and re-dissolved in 5 ml ethanol immediately. Twenty µl filtered sample (0.45micron filter paper) was injected into HPLC (Waters e2695 separation module, Alliance) equipped with a 2489 UV/visible detector (Waters) and eluted through a BDS Hypersil C18 column (250 x 4.6 mm, 5 µm). The mobile phase utilised was 250 ml Methanol:750 ml acetonitril, with a flow rate of 1.0 ml/min; and UV signal was detected at 264 nm. The run time for samples was 30 min. Vitamin D2 (98.7% pure) was used as standard (Sigma chemicals, Germany). The Empower software (version 3) was used for instrumental control, data acquisition, and analysis. Vitamin D2 content was reported as µg/g dry weight (DW) and the analysis was repeated at the end of 30, 60 and 90 days.

Determination of nutritional composition of button mushroom powder

The methods described by AOAC **[29]** were used to determine the moisture content, ash, crude protein, crude fat and crude fibre of button mushroom powder samples, with carbohydrate content being calculated by difference. Iron and calcium analysis was done by the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method **[29]** with some modifications. The measurements were carried out using the Perkin Elmer optima ICP- OES (Model:

OPTIMA 8300) at wavelengths 238.204 nm and 317.933 nm respectively.

Determination of antioxidant activity

Determination of total antioxidant capacity (TAC) by DPPH radical scavenging activity was done as per the method described by Tadhani *et al*. **[30]**. with some modifications. Accurately weighed (1.5g) sample was dissolved in 15 ml of 80 per cent methanol (pH 2.0) and continuously shaken for half an hour. The supernatant was decanted and the procedure was further repeated two times. The three supernatants were pooled, centrifuged for 15 min and filtered (Whatman No.1 filter paper, Sigma Aldrich, USA). Samples obtained were kept at -20°C till further analysis.

Different sample aliquots of 0.1 ml were taken in tubes and their final volume was made of one ml with methanol into which three ml DPPH reagent (15.77 mg of DPPH in 200 ml of methanol) was added. Tubes were vortexed and incubated in dark for 20 min at 37°C. Absorbance of the resulting oxidized solution was noted using a spectrophotometer (Shimadzu, UV-1800) at 517 nm against methanol taken as blank. Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) solution (10mg in 100ml distilled water) was taken as reference standard and a series of known concentration (5-20 µg) was prepared and treated the same as sample. The results were expressed as mg Trolox equivalent (TE)/100g.

Statistical analysis

All analyses were done in triplicates. The data obtained from the analyses of the button mushroom powder samples were expressed as mean ±standard deviation (SD) and subjected to analysis of variance (ANOVA) and where there was a significant difference ($p \leq 0.05$), means were separated using Tukey's Test. Statistical analysis was carried out with the use of Megastat in Excel in Windows 2016.

RESULTS AND DISCUSSION

Vitamin D2 enhancement

Vitamin D2 content was low in the control batch (C) mushroom powder. This finding is in line with other researches and may be related to the conditions of industrial mushroom growing, which often involves little exposure to sunlight **[26, 31, 32]**. Irradiation with sunlight led to a higher amount of vitamin D2 in button mushroom powders **(Table 1)** obtained after both the treatments (T-1 and T-2) with the highest value observed in T-1 (10.68±0.025 µg/g). Statistical analysis using one-way ANOVA revealed a significant ($p \leq 0.05$) enhancement in vitamin D2 content in button mushroom powders prepared by both the treatments. Further post hoc analysis using Tukey's test showed that there was a significant difference in T-1 and T-2, C and T-1 and C and T-2. Urbain &

Jacobsen reported vitamin D2 content in button mushroom slices after sun exposure to be 3.75 ± .86 μg/g DW which is lower than the values reported in the present study **[26]**. This could be because of the higher slice thickness (9mm) used in that study. It is worth mentioning here that the current study was carried out at a lower latitude (26.8°N vs 47.9 °N) and a lower altitude of 431 m, and hence with a higher natural UV-B irradiance. Similarly, much lesser vitamin D2 content in button mushroom, exposed to sunlight was reported by Simon *et al*. **[13]** compared to the results reported here. The reason for which could be use of whole mushrooms in that study. Mushroom slices upon sun exposure generate more vitamin D2 compared to whole mushrooms because of higher surface area to volume ratio and hence, higher ergosterol gets exposed and thinner slices yielded significantly higher amounts **[25, 26, 33]**. Amount of vitamin D2 enhanced further depends on the weather conditions, season, latitude, time of day, and exposure time. The gain in vitamin D2 value after one-hour exposure under overcast conditions was comparable to treatment for 15 minutes on a sunny day **[25]**.

In previous studies it has been found that the moisture content in mushrooms affects the vitamin D conversion efficiency **[34]**. KMS pre-treatment has significant effect on the drying time as well as the final moisture content of the product. As the pre-treatment level increases, the moisture uptake increases **[35]**. Lesser synthesis of vitamin D2 in T-2 could be attributed to higher moisture content in mushrooms dipped in KMS solution. The dilution effect of ergosterol at high moisture level results in a lower conversion rate to vitamin D2 **[12]**.

Nutrient content and antioxidant activity

Table 2 shows the nutrient composition and antioxidant activity of the prepared button mushroom powders. The highest level of moisture was seen in T-2 followed by T-1, however, the difference in both the treatments was not statistically significant ($p \leq 0.05$). As discussed earlier, KMS pre-treatment can increase the moisture content in the final product **[35]**. The least moisture was noted in control group.

No significant difference ($p \leq 0.05$) between the protein content of the treatment groups and the control was noted and mean values ranged between 29.96- 31.92g/100g. A protein content of 29 per cent was also observed by Ahlawat *et al* **[36]**. Another study also reported that pre-treatment with KMS solution does not significantly affect the protein content in button mushroom samples **[37]**. Further, the results of this study are consistent with the results described by Simon *et al*. **[13]**, where non-significant differences in protein content were noted in response to artificial UVB radiation or sunlight in button mushrooms as compared to control.

A significantly ($p \leq 0.05$) higher amount of ash content was noted in T-2. The reason behind high ash content could be due to dipping in KMS solution which could have added the mineral load. Different authors have reported variable ash percentages in button mushroom powder samples which ranged between 7.29- 12.99 g/100g **[38-40]**.

Fungal cell walls have dietary fibre components like hemicelluloses, chitins, mannans and beta-glucans which play an important role in some health attributes of mushrooms **[41]**. Although no significant difference ($p \leq 0.05$) between the fibre content was noted across the treatments, the control group had significantly lesser fibre. In another study conducted on milky mushrooms, the authors reported an increase in beta-glucan content upon exposure to sunlight for 90 minutes **[28]**.

Further, the control(C) group had a higher amount of fat (1.56g/100g). The results of the present study are in line with the fat percentage reported in button mushroom powder by Ahlawat *et al*. **[36]**. whereas other authors have reported values ranging between 2.22 – 5.26g/100g DW **[38, 40, 42]**. Statistical analysis revealed that difference was not significant between both the treatments, however, the difference was significant ($p \leq 0.05$) between C and T-1 and C and T-2. On the other hand, Simon *et al* **[13]** observed that the fatty acid content of the button mushrooms was not affected by exposure to sunlight or UVB light. There was no effect of the treatments on carbohydrate content of prepared button mushroom powders, the same being 46.83, 45.03 and 46.18g/100g DW in C, T-1 and T-2 respectively.

Non-significant difference in calcium content was noted between C and both the treatment groups and the mean calcium content ranged from 50.26 to 54.57 mg/100g. Goyal *et al.* reported a calcium content of 47.00 mg/100g in oven-dried button mushrooms **[43]**, on the contrary, Suleiman *et al.* reported much higher content of calcium in button mushroom powder **[38]**. Similarly, no significant effect of treatments was noted on the mean iron content of prepared button mushroom powders which ranged between 5.54 to 6.96 mg/100g. The mean iron content reported in other studies was higher ranging from 8.59 mg/100g to 30.99mg/100g **[36, 38, 40, 43]**. Mineral content varies according to diameter, type of substratum of fruiting body, and species of mushroom. Minerals present in the substrate are taken by growing mycelium and translocated to the fruit bodies **[43]**.

The effect of treatments on the Total Antioxidant Capacity (TAC) of button mushroom powder using DPPH Radical Scavenging Activity method was also investigated and the values of the same are presented in **Table 2**. Mushrooms from genera *Agaricus* have been reported to have the highest DPPH radical scavenging activity among the most commonly grown mushrooms **[39]**. In this study also all the button mushroom powder samples had high TAC. However, exposure to sunlight significantly decreased the TAC of both the treatment groups. A fall in DPPH value has also been reported by Galoti and Lavelli in UV exposed oyster and button mushrooms **[44]**. Compared to T-1, a lesser fall was seen in the T-2 group. Protective effect of vinegar and KMS on the phenolic compounds and antioxidant activity has also been reported **[37]**. Potassium bisulphite is a reducing agent and its presence in T-2 group might be the reason for lesser fall in DPPH value upon sun exposure.

Storage stability of Vitamin D2

A gradual fall in the vitamin D2 content was noted in all samples **(Table 3)** stored at ambient temperature over the period of three months. The initial value of vitamin D2 in control sample fell from 2.67±0.06 µg/g on day 1 to 2.25±0.08 µg/g over a period of 90 days exhibiting a fall of 15.73 per cent. The corresponding figures were 10.68±0.02 µg/g and 9.32±0.14 µg/g in the T-1 button mushroom powder with a fall of 12.73 percent. In a similar study, Slawinska *et al* reported that value of vitamin D2 in hot air-dried button mushrooms stored at room temperature for three months fell from 14.28 µg/g to 11.59 µg/g **[45]**. It was also noted that compared to dried oyster and shiitake mushrooms, largest decrease in vitamin D2 was seen in button mushrooms after 18 months of storage. Further, a fall of 6.86 percent in value of vitamin D2 was noted in T-2 sample in the current study. Unfortunately, there is a scarcity of publications available regarding the effect of KMS pre-treatment on storage stability of Vitamin D2 mushrooms. Statistical analysis revealed that fall in vitamin D2 content over a duration of 3 months was significant ($p \leq 0.05$) in all the categories. Further post hoc analysis showed that till 60 days fall in vitamin D2 content was not significant in any of the categories. Hence, it can be concluded that vitamin D2 content is relatively stable between 0-60 days at ambient and no appreciable losses were there.

Table 1: Effect of processing technique on vitamin D2 content (µg/g) *

Vitamin D2 µg/g	Control (C)	Treatment 1 (T-1)	Treatment 2 (T-2)
	2.67±0.06^{c}	**10.68±0.025^{a}**	**8.01±0.03^{b}**

*Values are on dry weight basis.

Mean and standard deviation with different letters within a row are significantly different ($p \leq 0.05$) calculated using one way ANOVA and post hoc Tukey's test.

Table 2: Mean nutrient content and antioxidant activity of button mushroom powder

NUTRIENT CONTENT			
Nutrient	**Control (C)**	**Treatment-1 (T-1)**	**Treatment-2 (T-2)**
Moisture (g/100g)	**3.30 ±0.09^{b}**	**4.25 ±0.03^{a}**	**4.32±0.09^{a}**
Protein (g/100g)	**31.92±0.05^{a}**	**31.75 ±0.15^{a}**	**29.96± 1.89^{a}**
Ash content (g/100g)	**10.14±0.06^{c}**	**10.83 ±0.08^{b}**	**11.77±0.02^{a}**
Crude fibre (g/100g)	**6.23±0.07^{b}**	**6.89±0.13^{a}**	**6.42 ±0.06^{a}**
Fat (g/100g)	**1.56 ±0.13^{a}**	**1.25±0.04^{b}**	**1.35 ±0.11^{b}**
Carbohydrate (g/100g)	**46.83 ±0.19^{a}**	**45.03 ±0.15^{a}**	**46.18 ±1.98^{a}**
Calcium (mg/100g)	**50.26±2.29^{a}**	**51.83±2.23^{a}**	**54.57 ±1.96^{a}**
Iron (mg/100g)	**5.54±0.76^{a}**	**6.32 ±0.20^{a}**	**6.96 ±0.03^{a}**
ANTIOXIDANT ACTIVITY			
TAC (mg TE/100g)	**95.48±0.13^{a}**	**92.07±0.47^{c}**	**93.43±0.10^{b}**

*Values are on dry weight basis
Mean and standard deviation with different letters within a row are significantly different ($p \leq 0.05$) calculated using one way ANOVA and post hoc Tukey's test.
TAC- Total Antioxidant Capacity
TE- Trolox Equivalents

Table 3: Mean Vitamin D2 content (µg/g)* of Button Mushroom powder over 3 months

Duration	**Control (C)**	**Treatment 1 (T-1)**	**Treatment 2 (T-2)**
Day 1	**2.67±0.06^{a}**	**10.68±0.02^{a}**	**8.01±0.03^{a}**
Day 30	**2.53±0.16^{a}**	**10.10±0.59^{ab}**	**7.93±0.11^{a}**
Day 60	**2.44±0.08^{ab}**	**9.97±0.26^{ab}**	**7.88±0.02^{a}**
Day 90	**2.25±0.08^{b}**	**9.32±0.14^{b}**	**7.46±0.24^{b}**

*Values are on dry weight basis.
Mean and standard deviation with different letters within a column are significantly different ($p \leq 0.05$) calculated using one way ANOVA and post hoc Tukey's test

CONCLUSION

Agaricus bisporus which is the most popular commercially grown mushroom species in the world generated nutritionally significant vitamin D2 after natural UV exposure to sunlight for a limited number of hours. The effect of both the treatments was limited to a significant increase in vitamin D2 content, without adversely affecting the nutritional properties. Both the treatment groups were able to retain good antioxidant properties. During storage at room temperature, a steady fall in vitamin D2 content was experienced in both the treatments, however, the overall quantity was still high. Given the widespread occurrence of vitamin D insufficiency worldwide, a sustainable solution is required to combat this persistent issue. Introduction of such kind of economical and consumer driven postharvest treatment for mushrooms can prove to be a wise move, without compromising the nutritional and nutraceutical quality parameters. Mushroom powder could be utilized as one of the promising ingredients for developing value-added products with improved nutritive value and nutraceutical properties. While natural UV exposure is a consumer-friendly approach, future research can be targeted upon fusing vitamin D enhancement with the commercial production line using artificial UV sources. After irradiation, the resulting powder can be standardised for vitamin D2 level that is suitable to meet the recommended dietary allowances.

ACKNOWLEDGEMENT

The authors would like to express thanks to the authorities of IIS (deemed to be University), Jaipur and Quality Control Laboratory at Amol Pharmaceuticals, Jaipur for providing facilities to carry out this research.

FUNDING SOURCE

The authors received no financial support for the research, authorship, and/or publication of this article.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Salemi S., Saedisomeolia A., Azimi F., Zolfigol S., Mohajerani E., Mohammadi M., Yaseri M. Optimizing the production of vitamin D in white button mushrooms (*Agaricus bisporus*) using ultraviolet radiation and measurement of its stability. LWT-Food Science and Technology, 2021;137:110401. http://dx.doi.org/10.1016/j.lwt.2020.110401

[2] Singh S., Joshi I. Button Mushroom - potential Source of vitamin D2 and possibilities of value addition.[Published online ahead of print] Current Nutrition and Food Science, 2023; 19(2):114-124 https://dx.doi.org/10.2174/1573401318666220614110008

[3] Capatina C., Carsote M., Caragheorgheopol A., Poiana C., Berteanu, M. Vitamin D deficiency in postmenopausal women– biological correlates. Maedica, 2014; 9(4): 316-322 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4316873/

[4] Sharma P., Mitra, P. Vitamin D: The need of the hour. Indian Journal of Clinical Biochemistry, 2016; 31(3); 243-244. http://dx.doi.org/10.1007/s12291-016-0578-5

[5] Parva N.R., Tadepalli S., Singh P., Qian A., Joshi R., Kandala H., Nookala V.K., Cheriyath P. Prevalence of vitamin D deficiency and associated risk factors in the US population (2011-2012). Cureus, 2018; 10(6):e2741. http://dx.doi.org/10.7759/cureus.2741

[6] Rhodes J. M., Sreedhar S., Eamon, L., Anne K.R. Low population mortality from COVID-19 in countries south of latitude 35 degrees North–supports vitamin D as a factor determining severity. Aliment Pharmacology Therapy, 2020; 51(12):1434-1437. http://dx.doi.org/10.1111/apt.15777

[7] Siddiqee M.H., Bhattacharjee B., Siddiqi U.R., Meshbahur R. M. High prevalence of vitamin D deficiency among the South Asian adults: a systematic review and meta-analysis. BMC Public Health, 2021;21(1): 1-18. https://doi.org/10.1186/s12889-021-11888-1

[8] Buttriss J.L., Lanham-New S.A. Is a vitamin D fortification strategy needed? Nutrition Bulletin, 2020; 45(2):115-122. https://doi.org/10.1111/nbu.12430

[9] Ross A.C., Taylor C.L., Yaktine A.L., Del Valle H.B. Dietary reference intakes for calcium and vitamin D. Washington (DC). Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. National Academies Press (US); 2011:13050. http://dx.doi.org/10.17226/13050

[10] Holick M.F. The vitamin D deficiency pandemic: a forgotten hormone important for health. Public Health Reviews, 2013;*32*(1); 267-283. https://doi.org/10.1007/BF03391602

[11] Harinarayan C.V., Holick M.F., Prasad U.V., Vani, P.S., Himabindu, G. Vitamin D status and sun exposure in India. Dermatoendocrinology, 2013; *5*(1); 130-141. http://dx.doi.org/10.4161/derm.23873

[12] Jiang Q., Zhang M., Mujumdar A.S. UV induced conversion during drying of ergosterol to vitamin D in various mushrooms: Effect of different drying conditions. Trends in Food Science and Technology, 2020; *105*: 200-210. http://dx.doi.org/10.1016/j.tifs.2020.09.011

[13] Simon R.R., Phillips K.M., Horst R.L., Munro I.C. Vitamin D mushrooms: comparison of the composition of button mushrooms (*Agaricus bisporus*) treated postharvest with UVB light or sunlight. Journal of Agricultural and Food Chemistry, 2011; 59(16):8724-8732. http://dx.doi.org/10.1021/jf201255b

[14] Cardwell G., Bornman J.F., James, A.P., Black L.J. A review of mushrooms as a potential source of dietary vitamin D. Nutrients, 2018;10(10):1498. http://dx.doi.org/10.3390/nu10101498

[15] Pereira E., Barros L., Martins A., Ferreira I.C. Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. Food Chemistry, 2012; 130(2): 394-403. http://dx.doi.org/10.1016/j.foodchem.2011.07.057

[16] Sinha S.K., Upadhyay T.K., Sharma, S.K. Nutritional-medicinal profile and quality categorization of fresh white button mushroom. Biointerface Research Applied. Chemistry, 2021; 11: 8669-8685. http://dx.doi.org/10.33263/BRIAC112.86698685

[17] Patel S., Goyal A. Recent developments in mushrooms as anti-cancer therapeutics: a review. 3 Biotech. 2012; 2(1): 1-15. http://dx.doi.org/10.1007/s13205-011-0036-2

[18] Thakur M. P., Singh H. K., Shukla C. S. 'Postharvest Processing, Value Addition and Marketing of Mushrooms', in M. Ahiduzzaman (ed.), Postharvest Technology - Recent Advances, New Perspectives and Applications, IntechOpen, London. 2022. https://doi.org/10.5772/intechopen.101168

[19] Singh N., Vaidya D., Mishra V., Thakur K.S. Shelf life and storage quality of white button mushrooms (*Agaricus bisporus*) as affected by packaging material. International Journal of Advanced Research, 2016; 4(11): 1790-1799. http://dx.doi.org/10.21474/IJAR01/2275

[20] Brennan M., Le Port G., Gormley R. Post-harvest treatment with citric acid or hydrogen peroxide to extend the shelf life of fresh sliced mushrooms. LWT-Food Science and Technology, 2000; 33(4):285-289. https://doi.org/10.1006/fstl.2000.0657

[21] Singh P., Langowski H.C., Wani A.A., Saengerlaub S. Recent advances in extending the shelf life of fresh *Agaricus* mushrooms: a review. Journal of the Science of Food and Agriculture, 2010; 90(9): 1393-1402. https://doi.org/10.1002/jsfa.3971

[22] Lagnika C., Zhang M., Nsor-Atindana J., Bashari M. Effects of

ultrasound and chemical treatments on white mushroom (*Agaricus bisporus*) prior to modified atmosphere packaging in extending shelf-life. Journal of Food Science and Technology, 2014; 51(12): 3749-3757. https://doi.org/10.1007/s13197-012-0904-8

[23] Nour V., Trandafir I., Ionica M.E. Effects of pretreatments and drying temperatures on the quality of dried button mushrooms. South Western Journal of Horticulture, Biology and Environment, 2011; 2(1):15-24. http://biozoojournals.ro/swjhbe/v2n1/02.swjhbe.v2n1.Nour.pdf

[24] NIFTEM. Mushroom Production Technology. Sonepat, India. National Institute of Food Technology Entrepreneurship and Management, 2021. http://niftem.ac.in/newsite/wp-content/uploads/2021/01/Mushroom-Production-technology.pdf

[25] Phillips K.M., Rasor A.S. A nutritionally meaningful increase in vitamin D in retail mushrooms is attainable by exposure to sunlight prior to consumption. Journal of Nutriion and Food Science, 2013; 3(6):1000236. http://dx.doi.org/10.4172/2155-9600.1000236

[26] Urbain P., Jakobsen J. Dose–response effect of sunlight on vitamin D2 production in *Agaricus bisporus* mushrooms. Journal of Agricultural and Food Chemistry, 2015; 63(37): 8156-8161. http://dx.doi.org/10.1021/acs.jafc.5b02945

[27] Nölle N., Argyropoulos D., Ambacher S., Müller J., Biesalski H.K. Vitamin D2 enrichment in mushrooms by natural or artificial UV-light during drying. LWT-Food Science and Technology, 2017; 85: 400-404. http://dx.doi.org/10.1016/j.lwt.2016.11.072

[28] Rathore H., Prasad S., Sehwag S., Sharma, S. Vitamin D2 fortification of *Calocybe indica* mushroom by natural and artificial UVB radiations and their potential effects on nutraceutical properties. 3 Biotech, 2020:10(2); 1-9. https://doi.org/10.1007/s13205-019-2024-x

[29] AOAC. Official Methods of Analysis of Association of Official Analytical Chemists. Edition No. 20. Washington DC. 2016.

[30] Tadhani M.B., Patel V.H., Subhash R. In vitro antioxidant activities of *Stevia rebaudiana* leaves and callus. Journal of Food Composition Analysis, 2007; 20(3-4): 323-329. http://dx.doi.org/10.1016/j.jfca.2006.08.004

[31] Phillips K.M., Ruggio D.M., Horst R.L., Minor B., Simon R.R., Feeney M.J., Byrdwell W.C., Haytowitz D.B. Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. Journal of Agricutural and Food Chemistry, 2011; 59(14): 7841-7853. http://dx.doi.org/10.1021/jf104246z

[32] Kristensen H., Rosenqvist E., Jakobsen J. Increase of vitamin D2 by UV-B exposure during the growth phase of white button mushroom (*Agaricus bisporus*). Food Nutrition Research, 2012; 56(1): 7114. http://dx.doi.org/10.3402/fnr.v56i0.7114

[33] Urbain P., Valverde J., Jakobsen J. Impact on vitamin D2, vitamin D4 and agaritine in *Agaricus bisporus* mushrooms after artificial and natural solar UV light exposure. Plant Foods for Human Nutrition, 2016; 71(3):314-321. https://doi.org/10.1007/s11130-016-0562-5

[34] Jasinghe V.J., Perera C.O. Ultraviolet irradiation: the generator of vitamin D2 in edible mushrooms. Food Chemistry, 2006; 95(4); 638-643. http://dx.doi.org/10.1016/j.foodchem.2005.01.046

[35] Nasiru M.M., Raj J.D. Yadav K.C. Effect of Potassium Metabisulphite and Temperature on Hot Air Drying of Dasheri Mango Slices. Science Letters, 2019; *7*(2); 91-98. http://thesciencepublishers.com/science_letters/files/v7i2-6-132019001-SL.pdf Published August 15,2019. Accessed on 29.01.23

[36] Ahlawat, O.P., Manikandan, K. and Singh, M. Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in *Agaricus bisporus* and *Volvariella volvacea*. Mushroom Research, 2016; 25(1):1-8. *https://www.researchgate.net/publication/309209418_Proximate_composition_of_different_mushroom_varieties_and_effect_of_UV_light_exposure_on_vitamin_D_con* tent_in_Agaricus_bisporus_and_Volvariella_volvacea Accessed 29.01.23

[37] Mutukwa I.B., Hall C.A., Cihacek L., Lee C.W. Evaluation of drying method and pretreatment effects on the nutritional and antioxidant properties of oyster mushroom (*Pleurotus ostreatus*). Journal of Food Processing and Preservation, 2019; 43(4); e13910. http://dx.doi.org/10.1111/jfpp.13910

[38] Sulieman A.A., Zhu K.X., Peng W., Shoaib M., Hassan H.A. Zhou H.M. Compositional, functional and pasting properties of composite flour fortified with button mushroom (*Agaricus bisporus*) powder and inulin. Journal of Food and Nutrition Research, 2017; *5*(8):614-621. http://dx.doi.org/10.12691/jfnr-5-8-12

[39] Reis F.S., Barros L., Martins A. Ferreira I.C. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. Food and Chemical Toxicology, 2012; 50(2); 191-197. http://dx.doi.org/10.1016/j.fct.2011.10.056

[40] Ishara J.R., Sila D.N., Kenji G.M., Buzera A.K. Nutritional and functional properties of mushroom (*Agaricus bisporus* & *Pleurotus ostreatus*) and their blends with maize flour. American Journal of Food Science and Technology, 2018; 6(1): 33-41. http://dx.doi.org/10.12691/ajfst-6-1-6

[41] Atila F., Owaid M.N., Shariati M.A. The nutritional and medical benefits of *Agaricus bisporus*: a review. Journal of Microbiology, Biotechnology and Food Science, 2017;7(3):281-286. http://dx.doi.org/10.15414/jmbfs.2017/18.7.3.281-286

[42] Tsai S.Y., Wu T.P., Huang S.J., Mau J.L. Non-volatile taste components of *Agaricus bisporus* harvested at different stages of maturity. Food Chemistry, 2007; 103(4):1457-1464. https://doi.org/10.1016/j.foodchem.2006.10.073

[43] Goyal R., Grewal R., Goyal R.K. Vitamin and mineral content of *Agaricus bisporus* (white button) and *Pleurotus sajor-caju* (dhingri) mushrooms. International Journal of Food Science and Nutrition,

2020;5:100-102. http://dx.doi.org/10.13140/RG.2.2.31004.10881

[44] Gallotti F. Lavelli, V. The effect of UV irradiation on vitamin D2 content and antioxidant and antiglycation activities of mushrooms. Foods, 2020; 9(8):1087. https://doi.org/10.3390/foods9081087

[45] Sławińska A., Fornal E., Radzki, W., Skrzypczak K., Zalewska-Korona, M., Michalak-Majewska M., Parfieniuk E., Stachniuk A. Study on vitamin D2 stability in dried mushrooms during drying and storage. Food Chemistry, 2016. 199:203-209. http://dx.doi.org/10.1016/j.foodchem.2015.11.131