STUDIES ON ENHANCEMENT OF VITAMIN D_2 IN OYSTER MUSHROOM (*Pleurotus eous*) USING UV RADIATION

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Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfillment of the requirements for the
Degree of

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in
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By

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UNIVERSITY OF AGRICULTURAL SCIENCES
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CERTIFICATE

This is to certify that the thesis entitled **"Studies on enhancement of vitamin D_2 in oyster mushroom (*Pleurotus eous*) using UV radiation"**, submitted in partial fulfillment of the requirements for the degree of **Master of science (Agriculture)** in **MICROBIOLOGY** to the University of Agricultural Sciences, Bangalore is a record of *bona fide* research work carried out by **Ms. PAVANI, B. C., PAMB 2256,** during the period of her study in this University, under my guidance and supervision. The thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

N. UMASHANKAR
(Major Advisor)

Place: Bengaluru
Month & Year: October, 2024

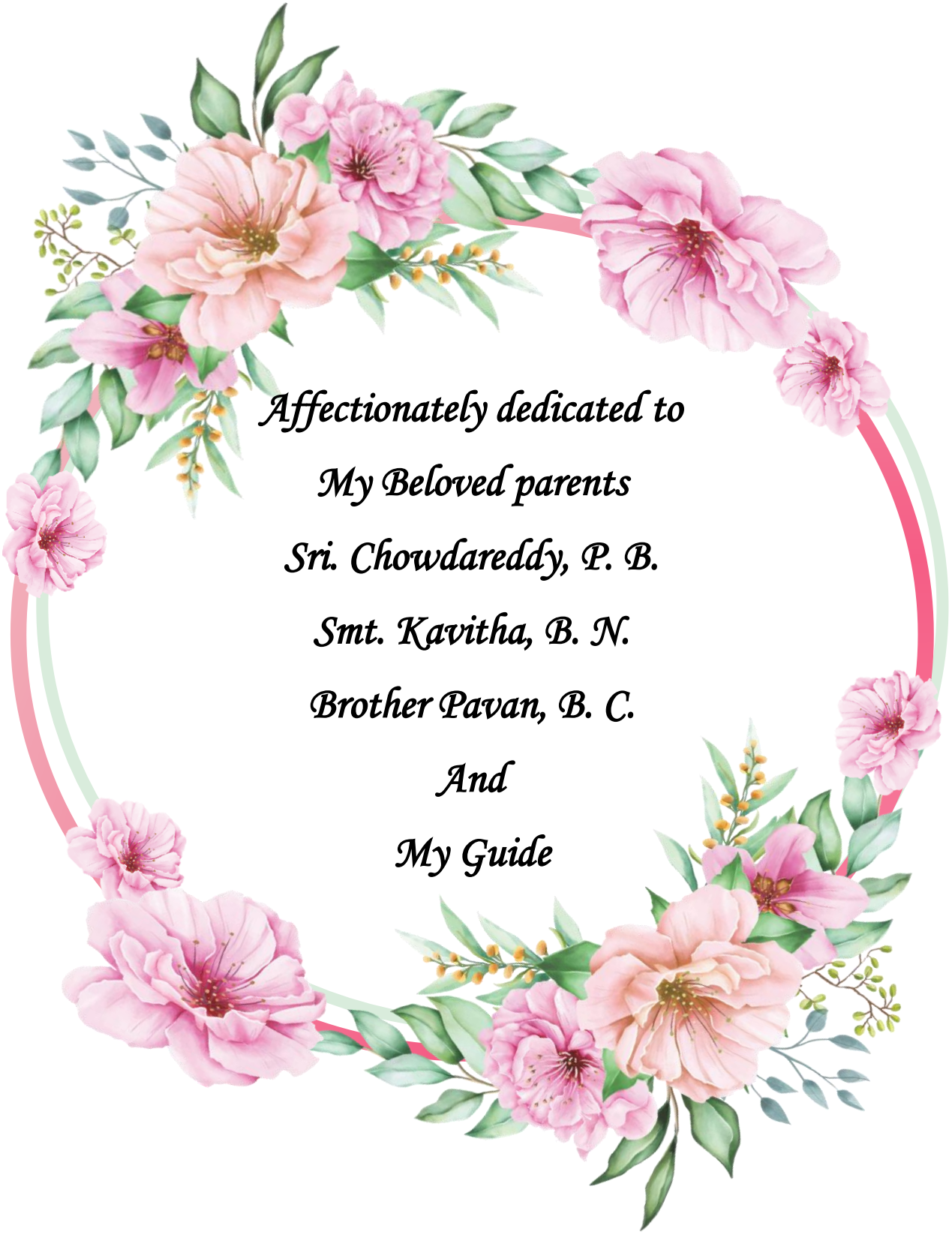
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2. _____
(MOHAN CHAVAN)

3. _____
(DARSHAN, M. B.)


Affectionately dedicated to
My Beloved parents
Sri. Chowdareddy, P. B.
Smt. Kavitha, B. N.
Brother Pavan, B. C.
And
My Guide

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With regardful memories.....

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Bangalore
October, 2024 ***Pavani, B. C.***

Fortification of Vitamin D_2 Content in Oyster Mushroom (*Pleurotus eous*) Using UV Radiation

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Introduction

- Vitamin D deficiency is a significant global health concern, particularly among vegetarians who lack sufficient dietary sources of vitamin D.
- Oyster mushrooms, are promising source of vitamin D_2 (ergocalciferol) due to their high ergosterol content. However, the natural vitamin D_2 content in mushrooms is often low or undetectable.
- Ultraviolet (UV) irradiation has been shown to efficiently convert ergosterol to vitamin D_2, making it a viable method to enhance the nutritional value of mushrooms.

Objective

To study the effect of UV radiation on Vitamin D_2 content in oyster mushroom

Materials and Methods

- Three forms of pink oyster mushrooms (*viz.*, whole, slice and powder) was exposed separately to UV radiation at different exposure time.
- Physical and chemical parameters *viz.*, physiological weight loss, vitamin D_2, protein, carbohydrates and minerals were analyzed (Jasinghe *et al.*, 2006).

Treatment details

Treatments	UV radiation exposure time (minutes)		
	Whole	Slice	Powder
T_1	0	0	0
T_2	10	10	10
T_3	20	20	20
T_4	30	30	30
T_5	40	40	40
T_6	50	50	50
T_7	60	60	60
T_8	70	70	70



Plate 1 : Pink oyster mushroom (*Pleurotus eous*)



Plate 2 : Exposure of whole mushroom to UV radiation



Plate 3 : Exposure of sliced mushroom to UV radiation



Plate 4 : Exposure of powdered mushroom to UV radiation

Results and Discussion

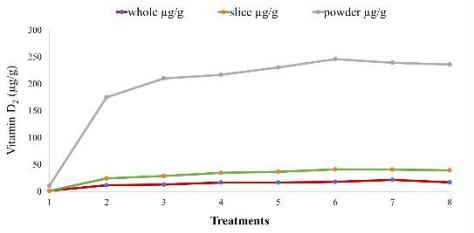


Fig. 1: Effect of UV radiation on vitamin D_2 content of oyster mushroom

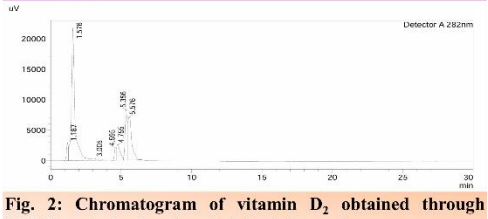


Fig. 2: Chromatogram of vitamin D_2 obtained through HPLC analysis of an irradiated whole mushroom

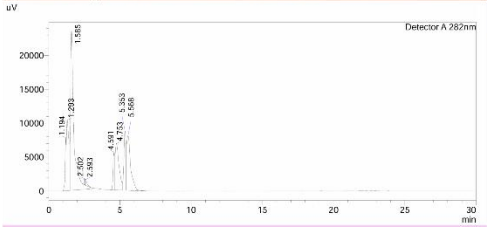


Fig. 3: Chromatogram of vitamin D_2 obtained through HPLC analysis of an irradiated sliced mushroom

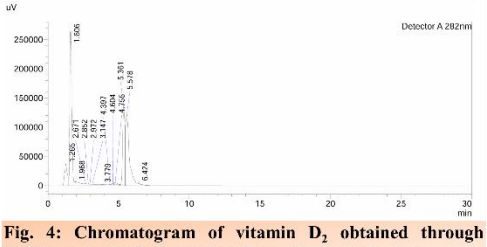


Fig. 4: Chromatogram of vitamin D_2 obtained through HPLC analysis of an irradiated powdered mushroom

- Vitamin D_2 content of UV irradiated mushroom was increased in all the treatments (T_2 to T_8) compared to control (T_1). However, among the different forms of mushroom exposed to UV, the maximum vitamin D_2 content was recorded in powdered form of mushroom (125.22 µg/g to 246.31 µg/g) compared to whole and sliced form.
- When mushrooms were exposed to UV radiation, their ergosterol was converted into vitamin D_2, resulting in a higher vitamin D_2 content compared to unexposed mushroom (Nolle *et al.*, 2017).
- The carbohydrate content and protein content was significantly on-par in all the treatments was observed.
- Weight loss was significantly increased from T_1 to T_8 due to dehydration.

Table 1: Effect of UV radiation on protein content (%) of oyster mushroom

Treatments	Whole	Slice	Powder
T_1	2.86^a	2.95^a	25.38^a
T_2	2.92^a	2.98^a	25.12^a
T_3	2.85^a	2.97^a	24.32^a
T_4	2.84^a	2.89^a	23.17^a
T_5	2.77^a	2.94^a	24.33^a
T_6	2.78^a	2.96^a	24.12^a
T_7	2.80^a	2.95^a	24.47^a
T_8	2.86^a	2.94^a	24.13^a

Table 2: Effect of UV radiation on carbohydrate content (%) of oyster mushroom

Treatments	Whole	Slice	Powder
T_1	3.45^a	3.78^a	32.60^a
T_2	3.47^a	3.81^a	32.62^a
T_3	3.48^a	3.80^a	31.48^a
T_4	3.43^a	3.81^a	31.06^a
T_5	3.43^a	3.83^a	30.82^a
T_6	3.44^a	3.83^a	31.84^a
T_7	3.46^a	3.82^a	30.62^a
T_8	3.43^a	3.82^a	31.10^a

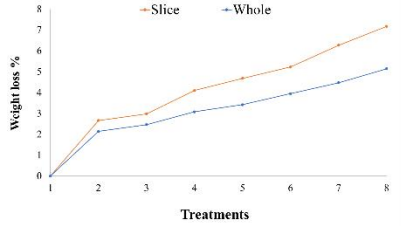


Fig. 5: Effect of UV radiation on weight loss (%) of oyster mushroom

Conclusion

The study was conducted to enhance vitamin D_2 in oyster mushroom upon exposure to UV light. Among all treatments, exposure duration of 50 & 60 min resulted in maximum conversion of ergosterol to vitamin D_2.

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JASINGHE, V. J. AND PERERA, C. O., 2006, Ultraviolet irradiation: the generator of vitamin D_2 in edible mushrooms. *Food chem.*, **95**(4): 638-643.

NOLLE, N., ARGYROPOULOS, D., AMBACHER, S., MULLER, J. AND BIESALSKI, H. K., 2017, Vitamin D_2 enrichment in mushrooms by natural or artificial UV-light during drying. *LWT-Food Sci. Technol.*, **85**: 400-404.

Advisory committee

Chairperson: Dr. N. Umashankar
Members: Dr. Nagaraju, K.
Dr. Mohan Chavan
Dr. Darshan, M. B.

STUDIES ON ENHANCEMENT OF VITAMIN D_2 IN OYSTER MUSHROOM *(Pleurotus eous*) USING UV RADIATION

PAVANI, B. C.

ABSTRACT

The vitamin D deficiency is significantly influenced by modern lifestyles and limited dietary sources around the globe. To meet the vitamin D requirement, exploring various strategies to increase vitamin D production is a need of hour. One such approach is fortifying foods, especially mushrooms, which contain ergosterol, a precursor of vitamin D which can be converted to vitamin D_2 upon UV exposure. Therefore, the present study investigates the enhancement of vitamin D_2 content in pink oyster mushrooms (*Pleurotus eous*) by exposing UV radiation. Three commercially available forms of pink oyster mushrooms (whole, sliced and powdered forms) were exposed to UV radiation for different time intervals (0, 10, 20, 30, 40, 50, 60 and 70 minutes) and various parameters were analysed. The maximum conversion of ergosterol to vitamin D_2 (whole: 21.95 µg/g, slice: 41.37 µg/g and powdered: 246.31 µg/g) was recorded, when mushroom was exposed to UV radiation for 50 min without significant changes in its nutritional properties. This exposure time (50 mins) was adopted further for storage studies to examine the retention of vitamin D_2 content, where UV irradiated whole and sliced mushroom was treated with 1% acetic acid as a preservative and stored at 4°C. During the storage, there was no significant loss of vitamin D_2 (whole: 18.38 µg/g and sliced: 41.45 µg/g) and other nutrients up to 6 days. Further, UV irradiated powdered mushrooms stored in refrigerated conditions retained vitamin D_2 for 30 days with minimal nutrient loss compared to mushroom stored at room temperature. The overall results indicate that exposure of mushroom under UV radiation for 50 minutes was sufficient to increase vitamin D_2 levels. Additionally, UV irradiated fresh mushrooms treated with 1% acetic acid and UV irradiated powdered mushrooms stored at 4°C, proved as an effective method for higher retention of vitamin D_2.

October, 2024

Dept. of Agricultural Microbiology
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(N. UMASHANKAR)
Major Advisor

UV ವಿಕಿರಣವನ್ನು ಬಳಸಿಕೊಂಡು ಚಿಪ್ಪು ಅಣಬೆಯಲ್ಲಿ (ಪ್ಲುರೋಟಸ್ ಇಯೊಸ್) ವಿಟಮಿನ್ D_2 ವರ್ಧನೆಯ ಕುರಿತು ಅಧ್ಯಯನ

ಪಾವನಿ, ಬಿ. ಸಿ.

ಸಾರಾಂಶ

ವಿಟಮಿನ್ ಡಿ ಕೊರತೆಯು ಆಧುನಿಕ ಜೀವನಶೈಲಿ ಮತ್ತು ಜಗತ್ತಿನಾದ್ಯಂತ ಸೀಮಿತ ಆಹಾರದ ಮೂಲಗಳಿಂದ ಗಮನಾರ್ಹವಾಗಿ ಪ್ರಭಾವಿತವಾಗಿದೆ. ವಿಟಮಿನ್ ಡಿ ಅಗತ್ಯವನ್ನು ಪೂರೈಸಲು, ವಿಟಮಿನ್ ಡಿ ಉತ್ಪಾದನೆಯನ್ನು ಹೆಚ್ಚಿಸಲು ವಿವಿಧ ತಂತ್ರಗಳನ್ನು ಅನ್ವೇಷಿಸುವುದು, ಆಹಾರಗಳ ಬಲವರ್ಧನೆ ಮಾಡುವುದು ಅವಶ್ಯಕವಾಗಿದ್ದು, ಅದರಲ್ಲಿ ವಿಶೇಷವಾಗಿ ಅಣಬೆಗಳು, ಎರ್ಗೊಸ್ಟೆರಾಲನ್ನು (ವಿಟಮಿನ್ D ಯ ಪೂರ್ವಗಾಮಿ) ಒಳಗೊಂಡಿರುವುದರಿಂದ, ಅಣಬೆಗಳನ್ನು UV ವಿಕರಣಕ್ಕೆ ಒಡ್ಡುವುದರಿಂದ ಎರ್ಗೊಸ್ಟೆರಾಲ್ ವಿಟಮಿನ್ D_2 ಆಗಿ ಪರಿವರ್ತನೆಗೊಳ್ಳುತ್ತದೆ. ಆದ್ದರಿಂದ, ಪ್ರಸ್ತುತ ಅಧ್ಯಯನವು UV ವಿಕಿರಣವನ್ನು ಬಳಸಿಕೊಂಡು ಚಿಪ್ಪು ಅಣಬೆಯಲ್ಲಿ (ಪ್ಲುರೋಟಸ್ ಇಯೊಸ್) ವಿಟಮಿನ್ D_2 ವರ್ಧನೆಯ ಕುರಿತು ತನಿಖೆ ಮಾಡಲಾಯಿತು. ವಾಣಿಜ್ಯಿಕವಾಗಿ ಲಭ್ಯವಿರುವ ಗುಲಾಬಿ ಬಣ್ಣದ ಚಿಪ್ಪು ಅಣಬೆಗಳ ಮೂರು ರೂಪಗಳನ್ನು (ಸಂಪೂರ್ಣ, ಸ್ಲೈಸ್ಡ್ ಮತ್ತು ಪುಡಿಮಾಡಿದ ರೂಪಗಳು) ವಿಭಿನ್ನ ಸಮಯದ ಮಧ್ಯಂತರಗಳಲ್ಲಿ (0, 10, 20, 30, 40, 50, 60 ಮತ್ತು 70 ನಿಮಿಷಗಳು) UV ವಿಕಿರಣಕ್ಕೆ ಒಡ್ಡಲಾಯಿತು ಮತ್ತು ವಿವಿಧ ನಿಯತಾಂಕಗಳನ್ನು ವಿಶ್ಲೇಷಿಸಲಾಯಿತು. ಅಣಬೆಯನ್ನು 50 ನಿಮಿಷಗಳ ಕಾಲ UV ವಿಕಿರಣಕ್ಕೆ ಒಡ್ಡಿದಾಗ, ಅದರ ಪೌಷ್ಟಿಕಾಂಶದ ಗುಣಲಕ್ಷಣಗಳಲ್ಲಿ ಗಮನಾರ್ಹ ಬದಲಾವಣೆಗಳಿಲ್ಲದೆ ಎರ್ಗೊಸ್ಟೆರಾಲ್ ವಿಟಮಿನ್ D_2 ಗೆ (ಸಂಪೂರ್ಣ: 21.95 µg/g, ಸ್ಲೈಸ್: 41.37 µg/g ಮತ್ತು ಪುಡಿ: 246.31 µg/g) ಗರಿಷ್ಟ ಪ್ರಮಾಣದಲ್ಲಿ ಪರಿವರ್ತನೆಯಾಗಿದೆ. ವಿಟಮಿನ್ D_2 ಅಂಶದ ಉಳಿಯುವಿಕೆ ಪರೀಕ್ಷಿಸಲು ಶೇಖರಣಾ ಅಧ್ಯಯನಗಳಿಗೆ ಈ ಮಾನ್ಯತೆ ಸಮಯವನ್ನು (50 ನಿಮಿಷಗಳು) ಅಳವಡಿಸಿಕೊಳ್ಳಲಾಯಿತು, ಅಲ್ಲಿ UV ವಿಕಿರಣಗೊಂಡ ಸಂಪೂರ್ಣ ಮತ್ತು ಸ್ಲೈಸ್ಡ್ ಅಣಬೆಯನ್ನು 1% ಅಸಿಟಿಕ್ ಆಮ್ಲದೊಂದಿಗೆ ಸಂಸ್ಕರಿಸಲಾಯಿತು ಮತ್ತು 4°C ನಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಯಿತು. ಶೇಖರಣೆಯ ಸಮಯದಲ್ಲಿ 6 ದಿನಗಳವರೆಗೆ, ವಿಟಮಿನ್ D_2 (ಸಂಪೂರ್ಣ: 18.38 µg/g ಮತ್ತು ಸ್ಲೈಸ್ಡ್: 41.45 µg/g) ಮತ್ತು ಇತರ ಪೋಷಕಾಂಶಗಳಲ್ಲಿ ಗಮನಾರ್ಹ ನಷ್ಟವಿಲ್ಲ. ಇದಲ್ಲದೆ, ಶೈತ್ಯೀಕರಿಸಿದ ಪರಿಸ್ಥಿತಿಗಳಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾದ UV ವಿಕಿರಣಗೊಳಿಸಿದ ಪುಡಿಮಾಡಿದ ಅಣಬೆಗಳು ಕೋಣೆಯ ಉಷ್ಣಾಂಶದಲ್ಲಿ ಶೇಖರಿಸಲಾದ ಅಣಬೆಗೆ ಹೋಲಿಸಿದರೆ ಕನಿಷ್ಠ ಪೋಷಕಾಂಶದ ನಷ್ಟದೊಂದಿಗೆ 30 ದಿನಗಳವರೆಗೆ ವಿಟಮಿನ್ D_2 ಅನ್ನು ಉಳಿಸಿಕೊಂಡಿವೆ. ವಿಟಮಿನ್ D_2 ಮಟ್ಟವನ್ನು ಹೆಚ್ಚಿಸಲು 50 ನಿಮಿಷಗಳ ಕಾಲ UV ವಿಕಿರಣದ ಅಡಿಯಲ್ಲಿ ಅಣಬೆಯನ್ನು ಒಡ್ಡಿಕೊಳ್ಳುವುದು ಸಾಕಾಗುತ್ತದೆ ಎಂದು ಒಟ್ಟಾರೆ ಫಲಿತಾಂಶಗಳು ಸೂಚಿಸುತ್ತವೆ. ಹೆಚ್ಚುವರಿಯಾಗಿ, 4 ° C ನಲ್ಲಿ ಶೇಖರಿಸಿಡಲಾದ 1% ಅಸಿಟಿಕ್ ಆಮ್ಲದೊಂದಿಗೆ ಸಂಸ್ಕರಿಸಿದ UV ವಿಕಿರಣಗೊಳಿಸಿದ ತಾಜಾ ಅಣಬೆಗಳು ಮತ್ತು UV ವಿಕಿರಣಗೊಳಿಸಿದ ಪುಡಿಮಾಡಿದ ಅಣಬೆಗಳು, ವಿಟಮಿನ್ D_2 ನ ಹೆಚ್ಚಿನ ಉಳಿವಿಕೆಗೆ ಪರಿಣಾಮಕಾರಿ ವಿಧಾನವೆಂದು ಸಾಬೀತಾಯಿತು.

ಅಕ್ಟೋಬರ್, 2024

ಕೃಷಿ ಸೂಕ್ಷ್ಮಜೀವ ಶಾಸ್ತ್ರ ವಿಭಾಗ

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಜಿ. ಕೆ. ವಿ. ಕೆ., ಬೆಂಗಳೂರು–65

(ಎನ್. ಉಮಾಶಂಕರ್)

ಪ್ರಮುಖ ಸಲಹೆಗಾರರು

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOLS	ABBREVIATIONS	SYMBOLS	ABBREVIATIONS
%	Per cent	OHD	Hydroxyvitamin D
g	Gram	nm	nanometer
mg	Milligram	DRI	Dietary Reference Intakes
µg	Microgram	d.w.	Dry weight
mL	Millilitre	f.w.	Fresh weight
L	Litre	psi	Pounds per square inch
cm	Centimeter	w/w	weight /weight
m	Meter	Kg	Kilogram
℃	Degree Celsius	ppm	Parts per million
UV	Ultraviolet	HPLC	High performance liquid chromatography
DM	Dry matter	µl	Microlitre
kcal	Kilocalories	rpm	Rotations per minute
et al.	Co-workers	W/m^2	Watt per square meter
i.e.	That is	J/cm^2	Joules per square centimeter
cfu	Colony forming unit	mW/cm^2	Milliwatts per square centimeter
viz.	Namely	mW/m^2	Milliwatts per square meter
Fig.	Figure	KCl	Potassium chloride

INTRODUCTION

I INTRODUCTION

Vitamin D is a group of fat-soluble secosteroids that play a vital role in enhancing the intestinal absorption of calcium, magnesium, and phosphate, along with other biological functions. Vitamin D is predominantly found in two distinct forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol), which exhibit structural similarities, with the sole exception of a methyl group located on carbon 24 and a double bond situated between carbons 22 and 23 (Keegan *et al*., 2013). Certain investigations indicate that vitamin D_3 is more effective in augmenting 25-hydroxy vitamin D status compared to vitamin D_2 (Logan *et al*., 2013; Balachandar *et al*., 2021), while a limited number of studies have asserted that both forms possess equivalent efficacy in sustaining serum 25-hydroxy vitamin D concentrations in humans (Biancuzzo *et al*., 2010; Phillips *et al*., 2011; Keegan *et al*., 2013; Chen *et al*., 2015).

Role of vitamin D in human health is well-established, as it plays a vital role in the prevention and treatment of numerous diseases. It is essential not only for bone health but has also garnered significant attention for its involvement in the treatment of diabetes, certain cancers, respiratory diseases, cardiovascular conditions, and autoimmune disorders. (Holick, 2005; Manson *et al*., 2019; Lips, 2021). Vitamin D deficiency has become a significant health issue, with reports indicating its prevalence across various countries, affecting individuals of all ages, genders, and ethnic backgrounds.

Currently, more than a billion people worldwide suffer from vitamin D deficiency. In India, the prevalence is estimated to be between 50% and 90% (Jan *et al*., 2019). Furthermore, approximately 30% of the population in the United States and about 40% of people in Europe are reported to have insufficient levels of vitamin D (Liu *et al*., 2018; Cashman *et al*., 2016). In Africa, more than 50% of the population experiences vitamin D deficiency (Keflie *et al*.,2015). Interestingly, despite abundant sunshine, several countries in the Middle East, such as the United Arab Emirates and Saudi Arabia, reported high rates of hypovitaminosis D, with deficiency rates of 50% and 59%, respectively (Green *et al*., 2015).

Among the dietary sources, vitamin D_3 is found predominantly in animal-derived foods, rich sources include fatty fish like salmon and trout, fish liver oils, egg yolks, and fortified foods. In contrast, vitamin D_2 is found in some phytoplankton, yeast and mushrooms (Taofiq *et al*., 2017). The primary source of vitamin D_3 for most individuals is direct sunlight, which initiates the synthesis of vitamin D from its precursor, 7-dehydrocholesterol, found in the skin. (Holick, 2002). The production appears to be inadequate owing to lifestyle changes leading to the limited sun exposure, large use of sunscreens (Holick, 2010).

The dietary origins of vitamin D are predominantly confined to some fishes or cod liver oil. Certain nations engage in the fortification of specific food items, such as milk, orange juice, as well as various cereals and yogurts, with vitamin D (Tangpricha *et al*., 2003). Nevertheless, these initiatives prove insufficient to tackle vitamin D deficiency. Thereby, there is a need of exploration of alternative strategies to fulfill the requisite vitamin D levels. Furthermore, the transition to supplementation is not deemed a satisfactory resolution, such supplements may not be uniformly accessible and the challenge of vitamin D deficiency remains a global concern. Moreover, the extended consumption of vitamin D supplements may potentially result in deleterious health consequences, including hypercalcemia, hyperphosphatemia, hypercalciuria, and intoxication (Razzaque, 2018). Consequently, alternative methodologies are currently under investigation to satisfy the demand for vitamin D (Calvo and Whiting, 2013). In recent years, a variety of strategies have been employed to attain the recommended daily intake of vitamin D. According to the Indian Council of Medical Research (ICMR-NIN, 2020), the prescribed daily intake of vitamin D is established at 600 IU.

Among the well-known sources of vitamin D, edible mushrooms are the only vegetarian source popular for their delicacy, nutritional and therapeutic values among both vegetarians and non-vegetarian population groups. However, cultivated mushrooms typically contain little or no vitamin D_2, though they are rich in ergosterol, the precursor to vitamin D_2 (Cardwell *et al*., 2018). Under UV radiation, the B ring of ergosterol undergoes photochemical cleavage, leading to the formation of an intermediate called pre- vitamin D_2. This intermediate then undergoes thermal rearrangement to form vitamin D_2

(ergocalciferol). However, pre- vitamin D_2 can also absorb UV light, and prolonged exposure may result in the production of undesirable byproducts, such as tachysterol and lumisterol, through photoisomerization (Jashinge and Perera, 2006).

There are three types of UV radiation *viz*., UV-A (315 nm to 400 nm), UV-B (280 nm to 315 nm) and UV-C (190 nm to 280 nm) (Teichmann *et al*., 2007). Among them, UV-C treated mushrooms displayed reduced color changes, lower tissue electrolyte leakage, and decreased levels of MDA (Melon dialdehyde), while maintaining higher soluble solid content, soluble protein, CAT (Catalase), PAL (Phenylalanine ammonia lyase) activities, and overall quality compared to untreated mushrooms. Additionally, UV-C treatment demonstrated potential for surface decontamination and holds promise for extending the shelf life of oyster mushrooms (Wang *et al*., 2017a).

Mushrooms are known to contain ergosterol, which serves as a precursor for vitamin D_2; the highest concentration of ergosterol was observed in button mushrooms (7800 ± 360 µg g-1 DM), followed by shiitake mushrooms (6050 ± 70 µg g-1 DM), oyster mushrooms (4800 ± 80 µg g-1 DM), abalone mushrooms (4350 ± 160 µg g-1 DM), with enoki mushrooms exhibiting the lowest content (680 ± 140 µg g-1 DM). The distribution of ergosterol varied significantly across different fungal tissues, with the gills exhibiting the highest concentration and the stems displaying the lowest levels (Jashinge and Perera, 2005).

Mushrooms are reported to contribute to a holistic nutritional regimen, encompassing all requisite nutrients and several nutraceutical constituents such as polysaccharides, terpenes, antioxidants, and biologically active proteins. Furthermore, they exhibit a wealth of various restorative compounds, including β-glucans, tocopherol, and terpenoids, which impart them as a significant therapeutic potential for the prevention and treatment of numerous degenerative illnesses (Roncero-Ramos *et al*., 2017; Ma *et al*., 2018). Mushrooms that are abundant in vitamin D are regarded as functional foods, owing to their promise as a treatment for various degenerative conditions, including osteoporosis, diabetes, cancers, and cardiovascular diseases (Urbain *et al*., 2011).

The mushroom with the widest culinary acceptance and the highest price is the oyster mushroom, or *Pleurotus* spp. The oyster mushroom is the third-most produced and consumed mushroom in the world. *Pleurotus eryngii, Pleurotus citrinopileatus, Pleurotus flabellatus, Pleurotus ostreatus, Pleurotus djamor* var. *roseus, Pleurotus eous, Pleurotus florida* and other species have all been reported as being grown in captivity (Raman *et al*., 2021). *Pleurotus* spp. can be cultivated on a variety of wastes, weeds and by products from agroforestry to make food, feed, medicines, enzymes, or to break down and detoxify waste. Along with considerable amounts of protein, fiber and minerals, it contains all nine of the necessary amino acids that humans require (Wang *et al*., 2017b).

Pleurotus spp. contain a significant level of ergosterol, and there are no investigations on *Pleurotus eous* in terms of vitamin D_2. Thus, the current study focusing on to explore the effect of UV radiation on the conversion of ergosterol to vitamin D_2, which results in an increase in vitamin D_2 content in all three commercially available forms of *Pleurotus eous* (whole, sliced, and powdered) mushrooms, also the retention of vitamin D_2 in UV-induced oyster mushrooms during storage period was tested with the following objectives:

1. To study the effect of UV radiation on vitamin D_2 content in oyster mushroom
2. To study the retention of vitamin D_2 content in UV radiation induced oyster mushroom during storage

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

This chapter deals with the review of relevant literature pertaining to various parameters involved in the present investigation, which were referred as footprints to follow the guidelines. Studies conducted by researchers in India and abroad have been reviewed for importance of vitamin D and its sources, status, cultivation, nutritional and medicinal value of mushroom, effect of UV radiation on vitamin D content and other nutrients present in the mushroom.

2.1 Importance of vitamin D

Vitamin D plays a crucial role in various metabolic processes, including the promotion of calcium and phosphorus metabolism, as well as the maintenance of neuromuscular and skeletal homeostasis. More recently, various non-skeletal diseases have been associated with vitamin D deficiency such as cancer, diabetes, heart disease, neuropsychiatric disorders and other chronic conditions. Furthermore, insufficient levels of vitamin D can disrupt the functions of the pancreas and immune system, leading to a range of health issues (Leuven, 2020).

Vitamin D is primarily obtained through endogenous synthesis following exposure to ultraviolet light from direct sunlight, as well as from dietary sources and supplements (Elangovan *et al*., 2017). Deficiency in vitamin D most commonly leads to rickets and osteomalacia, conditions characterized by impaired mineralization of calcium and phosphorus. Sufficient vitamin D_3 (Cholecalciferol) levels are also linked to healthy muscle function, protection against cardiovascular disease, neurodegenerative diseases, children's respiratory illnesses, some cancers, and type 1 and type 2 diabetes, though there is currently conflicting evidence regarding non-skeletal benefits (Cardwell *et al*., 2018).

Among the various forms of vitamin D, the two most biologically active/relevant are cholecalciferol (vitamin D_3), which is produced when skin is exposed to solar radiations and ergocalciferol (vitamin D_2) which is formed from the plant steroid called ergosterol, by UV irradiation. (Chen *et al*., 2015). It is important to mention that both forms of vitamin D are reported to have equal efficacy to maintain serum levels of 25-OHD. Vitamins D_2

and D_3 have very similar structures except that vitamin D_2 has one more double bond and a methyl group compared with vitamin D_3.

Vitamin D deficiency has become a significant health issue, with reports indicating its prevalence across various countries, affecting individuals of all ages, genders, and ethnic backgrounds. Currently, more than a billion people worldwide suffer from vitamin D deficiency. In India, the prevalence is estimated to be between 50 per cent and 90 per cent (Jan *et al*., 2019). Furthermore, approximately 30 per cent of the population in the United States and about 40 per cent of people in Europe are reported to have insufficient levels of vitamin D (Liu *et al*., 2018; Cashman *et al*., 2016). In Africa, more than 50 per cent of the population experiences vitamin D deficiency (Keflie *et al*.,2015). Interestingly, despite abundant sunshine, several countries in the Middle East, such as the United Arab Emirates and Saudi Arabia, report high rates of hypovitaminosis D, with deficiency rates of 50 per cent and 59 per cent, respectively (Green *et al*., 2015).

In India, where a significant portion of the population follows a vegetarian diet, the intake of vitamin D from dietary sources is notably low. Vitamin D-rich foods are often expensive and limited, making them less accessible, especially for vegetarians. Similarly, vitamin D supplements are costly and not a feasible option for the general population. To address vitamin D deficiency more effectively, fortifying widely consumed staple foods with vitamin D is a practical solution. Milk, being one of the few vegetarian sources of vitamin D, should be fortified, though this is not commonly done in India. Enhancing the nutritional content of staple foods is a more economical and accessible strategy compared to supplementation, particularly for large groups such as women, pregnant and lactating mothers, infants, children and teenagers.

2.2 Sources of vitamin D

According to Indian Council of Medical Research (ICMR-NIN, 2020), the recommended daily intake of vitamin D is 400 IU or 10 µg for infants, 600 IU or 15 µg for children and adults, and 800 IU or 20 µg for old age group. There are several ways to meet the recommended daily intake of vitamin D, such as exposure to sunlight, supplements and ingestion of some natural or fortified foods containing vitamin D**.**

2.2.1 Sunlight as a Source of Vitamin D

Vitamin D can be synthesized in the skin from 7-dehydrocholesterol when exposed to UV rays from sunlight. However, this source is dependent on weather and seasons, and it diminishes during periods with insufficient sunlight. As a result, alternative sources, such as dietary supplements or fortified foods, are often necessary (Jakobsen, 2007). In recent years, widespread avoidance of sunlight due to behavioral and cultural factors, as well as the increased use of sunscreen, has further hindered the body's ability to produce vitamin D (Spiro and Buttriss, 2014).

2.2.2 Naturally Occurring Food Sources of Vitamin D

Natural food sources of vitamin D are very few, vitamin D_3 can be obtained from dietary sources and supplements *viz.,* eggs, butter, milk, and dairy products, as well as liver and other meats, can provide a small quantity of vitamin D_3. Higher concentrations of vitamin D_3 can be found in animal sources such as, cod liver oil, tuna, salmon, mackerel, sardines, herring, trace sole, cod, eggs and beef liver. The levels vitamin D varies from source-to-source e.g., in cod liver oil, the vitamin D content is 250 µg/100 g and in fatty fish salmon or mackerel the vitamin D content ranges from 8-30 µg/100 g, while as levels in lean fish such as halibut, sole and tuna is 3-9 µg/100 g (Pludowski *et al*., 2023). So, the vegetarians are at high risk of vitamin D deficiency disorders (like obesity, heart diseases, cancers, diabetes, arthritis etc.) because it is found in very few plant-based foods, fortified foods like plant-based milk and cereals, but they may not provide enough vitamin D_2 to meet daily requirement (Jashinge and Perera, 2005). Hence, the vegetarian source of vitamin D_2 (Ergocalciferol) from mushroom plays a vital role.

2.3 Mushroom

Mushrooms are saprophytic fungi, a class of primitive plants that lack chlorophyll and obtain their food requitement from decomposed organic material (Bano *et al*., 1992). In nature they occur in open fields, meadows, marsh edges, garden and farmyards, lawns and hardwood forests under shade i.e. found growing on dead and decaying/live plants (debris). These mushrooms on earth differ greatly in shape, size, colour, form and structure.

Chinese were first to describe cultivation methods of mushrooms. The word 'mushroom' is thought to be derived from the French word 'Mousseron', 'mouse' or moss, while in India it is known as Ksumpa in Sanskrit and Khumbi or Kukurmutta in Hindi. The method of cultivation was recorded as early as 300 BC and their intentional cultivation was started as early as 600 AD in China (Singh *et al*., 2007). Its cultivation originated in Paris (France) during 1650 and was later extended to European countries. The French botanist Tournefort described its cultivation method in 1707. Grain spawn was introduced in 1932 by Sinden in USA. In India, cultivation of mushroom started in 1943 when paddy straw mushroom was cultivated for the first time at coimbatore. However, commercial production of mushroom started at Chail (Himachal Pradesh) during 1966 by the Maharaja of Patiala (Tewari, 1976).

Although, many species of mushroom are edible but only three types, viz white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* Spp.) and paddy straw mushroom (*Volvariella volvacea*) are grown commercially in India depending upon the suitability of season (Khadar and Pandya, 1981). The cultivation of mushroom requires low temperature so it was mostly grown in hilly region but with the advent of modern cultivation technology it is now possible to cultivate mushroom seasonally under controlled condition and throughout the year by employing environmentally controlled conditions. An approach for cultivation of mushroom (*Pleurotus florida*) by using two different agricultural wastes in laboratory condition was experimented by Narayanasamy *et al*. (2009). Mushroom packs or mushroom beds with paddy straw and cotton as a substrates were put in the lab and maintained temperature at 23 ℃ and humidity 80 per cent for 4 and 7 weeks for paddy straw and cotton respectively. The authors reported that an average yield of ½ kg/pack was obtained in 4 weeks in paddy straw and 1 kg was obtained in cotton waste after 7 weeks. So, cotton waste gave a greater number of mushrooms compared to paddy straw mushroom. Hassan *et al*. (2010), studied the cultivation of the king oyster mushroom (*Pleurotus eryngii*) in Egypt by using some local lignocellulosic wastes as growing media. They concluded that cultivation of *Pleurotus eryngii* under local conditions and using the available cheap lignocellulosic wastes in Egypt

was an important achievement, since this type of mushroom was highly prized for their nutritive and medicinal value.

The potential use of rice waste lignocellulose and its amendments as substrate for the cultivation of *Pleurotus eous* Strain P-31 in Ghana was evaluated by Wiafe-kwagyan *et al*. (2016). The effect of raw untreated rice straw and pre-treated straw (composted for 0, 4, 8 and 12 days) supplemented with either 5, 10 and 15 per cent rice bran and 1 per cent $CaCO_3$ on growth rate, yield performance and biological efficiency (BE) of *Pleurotus eous* Strain P-31 was investigated using the conventional pasteurized bagged technique. Telang *et al*. (2010) evaluated the different substrate (soyabean, wheat, jowar, tur, paddy, bajra and sunflower) for identifying the yield, biological efficiency and proximate composition of *Pleurotus eous*.

Since ancient times mushrooms have been recorded as an important food item and have been acknowledged because of their nutritional values and therapeutic properties. There are about 12,000 species of such mushrooms that exist worldwide. Of these, only 2000 species are considered to be edible (Rathore *et al*., 2017). Currently, only about 35 mushroom species are cultivated commercially, worldwide and 21 species are cultivated on an industrial scale (Muhammad and Suleiman, 2015).

White button mushrooms (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp), and paddy straw mushroom (*Volvariella volvacea*) are three commonly grown species of edible mushrooms. World production increased to 43 million tons in 2018-19 (Singh *et al*., 2010) and may surpass 50 million tons by 2025. *Agaricus bisporus* (button mushroom) still retains the highest place overall world production. In today's time China is leading in global mushroom production and produces around 93 per cent of world mushroom production in 2020. The second largest in mushroom production is Japan, followed by USA (FAO, 2020). India ranks at number 6^{th} in the production of edible mushrooms.

India is a country with its diverse agro-climatic conditions ranging from tropical to sub-tropical and temperate regions, offers an ideal environment for mushroom cultivation. The favorable climate, abundant farm residues, affordable labor, and the demand for

protein-rich mushrooms in a largely vegetarian population create a perfect setting for mushroom farming. In recent years, India has focused on producing a variety of mushrooms suited to its different climatic zones. These include the temperate *Agaricus bisporus,* sub-tropical species like *Pleurotus, Hypsizygus ulmarius,* and *Lentinula edodes,* as well as tropical mushrooms such as *Volvariella volvacea* and *Calocybe indica* (Verma, 2014).

2.2 Nutritional and medicinal value of mushroom

Mushrooms have traditionally been used for medicinal and tonic properties for treating hepatitis and other diseases of liver and for promoting longevity and cosmetic products such as skin whitening agent and have been reported to have anti-fungal and anti-bacterial properties (Chang and Buswell, 1996). Mushrooms are highly nutritious, low-calorie foods that provide high-quality proteins, vitamins and minerals. They serve as a significant source of both food and medicine. Traditional knowledge has long recognized the medicinal value of both edible and wild mushrooms and contemporary research is exploring their bioactivity for various health conditions (Selima Khatun *et al*., 2012). Due to the presence of a huge amount of nutraceutical components mushrooms are well-recognised functional foods. Mushrooms are consumed mostly due to their nutritional value and sometimes also due to their medicinal values. The nutritional importance of mushrooms such as low fat, high protein, and low energy contents are well recognized. Mushrooms are also rich in minerals such as iron and phosphorus, as well as in vitamins like thiamine, riboflavin, niacin, ascorbic acid and ergosterol.

They contain polysaccharides primarily β-glucans and glycoproteins and bioactive constituents like (terpenoids, alkaloids, acids, lactones, polyphenolic compounds, sesquiterpenes, sterols, vitamins, nucleotide analogues and metal chelating agents). According to certain findings, immune system activation explains mushroom's anticancer effect (Meng *et al*., 2016) and immunomodulatory (Tanaka *et al*., 2016) actions. Due to the presence of biologically active substances, mushrooms can serve as anti-viral, anti-cancer, immune potentiating, hepatoprotective and hypo-cholesterolemic agents. The properties like low in fat and high in fiber contents give mushrooms a great potential to prevent

cardiovascular diseases, also being rich source of natural antioxidants, they help in reducing oxidative damages. Mushrooms being rich sources of fiber, and nutrients such as proteins, minerals, vitamins with lower amounts of fats and calories have been utilized for centuries by many cultures (Roncero-Ramos *et al*., 2017). Bano *et al*. (1988) reported that mushroom composed of linoleic acid which reduces the risk of coronary heart disease because of high dietary fibre content (7.4 to 27.66 per cent on dry weight basis) and also recommended for diabetic patients. It also reduces risk of colon cancer.

Chang and Miles (1989) reported that mushroom consists of protein (10-40 per cent), carbohydrate (13-70 per cent), fat (1-8 per cent), minerals and significant amounts of essential amino acids. Mineral content of mushroom indicated that 10 per cent ash (dry weight basis) contains potassium, phosphorus, sodium, calcium and magnesium. Anonymous (2006) reported that mushroom is excellent source of high-quality proteins (14 to 14 per cent) compared to most vegetables. Mushroom also contains total amino acids (25-40 per cent) and they are good sources of vitamins (vitamin B like riboflavin, niacin, thiamin, pantothenic acid and ascorbic acid) and minerals (calcium, phosphorus and potassium are present in fair quantity along with copper, iron) all of which are essential for formation of bones, teeth and good eyesight. They also reported that mushrooms are low in sodium and high in potassium and therefore considered as an ideal food for persons suffering from high blood pressure.

Mushrooms on fresh weight basis have a nutritional value of 90-93 per cent moisture, on dry weight basis it contains, 28-42.5 per cent crude protein, 8.3-16.2 per cent crude fiber, 9.4-14.5 per cent ash, 40-59.4 per cent carbohydrate and 3.1-3.8 per cent fat. Mineral content per 100 g of dry weight is 71 mg of calcium, 912 mg of phosphorus, 106 mg of sodium, 8.8 mg of iron and 2850 mg of potassium. Each 100 g of mushrooms contains 0.16 mg of thiamine (B_1), 2.4 mg of riboflavin (B_2) and 54.30 mg of niacin (B_3) (Mehta *et al*., 2011).

Mushrooms contains ergosterol, a precursor of vitamin D_2, the highest ergosterol content was found in button mushrooms (7800 ± 360 µg g-1 DM), followed by shiitake mushrooms (6050 ± 70 µg g-1 DM), oyster mushrooms (4800 ± 80 µg g-1 DM), abalone

mushrooms (4350 ± 160 µg g-1 DM) and enoki mushrooms (680 ± 140 µg g-1 DM) was the lowest. The Ergosterol distribution was significantly different in different tissues of mushrooms, the highest content in gills and the lowest content in stems (Jashinge and Perera, 2005). The UV exposure breaks the B-ring at C9 and C10 of ergosterol to form pre-vitamin D_2, which undergoes thermally induced rearrangement to vitamin D_2 (Fig. 1).

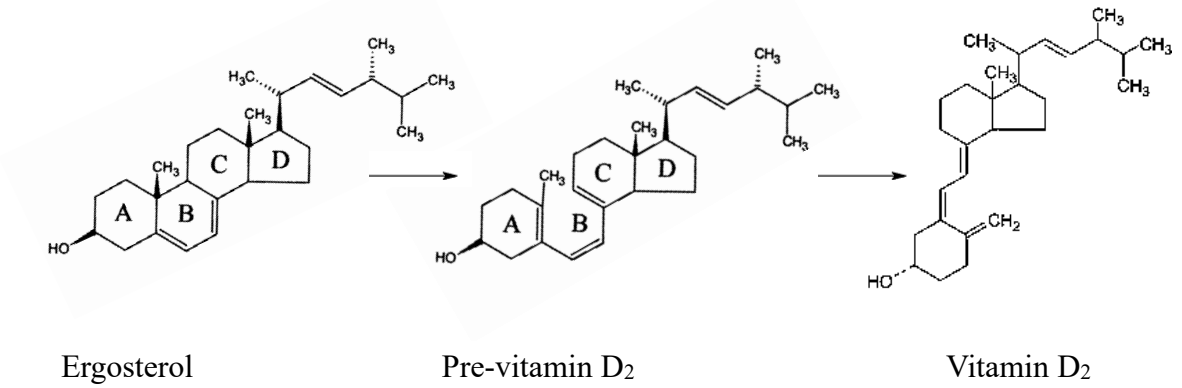


Fig. 1. Mechanism of conversion of ergosterol to vitamin D_2

Pleurotus eous was grown on different agro wastes viz., soybean, paddy, wheat, jowar, bajra, tur and sunflower straw to study the effect of these agro waste on yield, bio-efficiency and proximate composition of mushroom, results revealed that *P. eous* on paddy straw showed maximum stipe weight (1.10 g), Ca (360 mg/100 g) content whereas bajra straw showed maximum pileus weight (5.98 g). Maximum yield (820.33 g/kg dry straw) with 82.03 per cent biological efficiency, protein (30.50 per cent), crude fiber (9.00-12 per cent), ash (6.50-10.52 per cent), P (965 mg) and Fe (15.60 mg) content of mushroom were observed with soybean straw. Jowar straw favoured to show highest moisture (91.21 per cent) content, wheat straw showed maximum fat (2.62 per cent) content and sunflower straw showed maximum carbohydrate content (52.00 per cent) in mushroom fruiting bodies (Telang *et al*., 2010; Wiafe-kwagyan *et al*., 2016).

Many comparative studies had been carried out between various mushroom varieties to know about the nutritional and health benefits of mushroom in a broader sense. Benjamin and Anuradha (1991) reported that *Agaricus bisporus* had higher protein, ascorbic acid, ash and calcium content, whereas *Pleurotus* species were rich in thiamine,

riboflavin, fibre, sodium and potassium. Poongkodi and Sakthisekaran (1995) reported that mushrooms had more protein (20 to 35 per cent) than any other vegetable or fruit and also rich source of vitamins including riboflavin, niacin, folic acid, thiamine, pyridoxine, ascorbic acid and α-tocopherol. They are opinioned that high proteins, sterols, micro-elements and low caloric contents make mushrooms ideal for prevention of cardiovascular diseases.

Khan *et al*., (2008) found out the nutritional composition of six species of oyster mushrooms such as *Pleurotus sajor-caju*, *P. ostreatus*, *P. florida*, *P. cystidiosus*, *P. highking* 51 and *P. geestaranus*. They also reported that, the moisture content of fresh oyster mushrooms was 85-88 per cent. The protein content was highest in *P. sajor-caju* (24.5 g/100 g of dry weight) followed by *P. ostreatus, P. highking* 51*, P. florida, P. geestaranus* and *P. cystidiosus*. The highest lipid content was recorded in *P. cystidiosus* (5.5g/100 g dry sample) followed by *P. highking* 51*, P. sajor-caju, P. florida, P. geestaranus* and *P. ostreatus*. The carbohydrate content was highest in *P. geestaranus* (45.9 g/100 g dry sample) followed by *P. cystidiosus, P. florida, P. ostreatus, P. sajor-caju* and *P. highking* 51. The fiber content was maximum in *P. highking* 51 (30.3 g/100 g dry sample) followed by *P. ostreatus, P. florida, P. geestaranus, P. sajor-caju* and *P. cystidiosus*. The total ash content was highest in *P. florida* (8.3 g/100 g dry sample) followed by *P. sajor-caju, P. ostreatus, P. cystidisus, P. highking* 51 and *P. geestaranus*. Following these data, the highest metabolizable energy was found in *P. cystidiosus* (262.8 kcal/100 g dry sample) followed by *P. Sajor-caju* (254.1 kcal/100 g), *P. geestaranus* (252.7 kcal/100 g), *P. florida* (250.1 kcal/100 g), *P. Highking* 51 (249.7 kcal/100 g) and *P. ostreatus* (242.6 kcal/100 g).

2.4 Mushrooms as a Source of Vitamin D

Mushrooms are a valuable source of vitamin D_2, which is essential for the body's structure and function. In a study, pre-harvest *Agaricus bisporus* mushrooms were exposed to UV-B and UV-C light (254 and 312 nm) for various durations to enhance their vitamin D_2 content. After three days, the results showed increased levels of vitamin D_2 at all irradiation times. Notably, shorter irradiation periods resulted in higher concentrations of vitamin D_2 compared to longer exposure times (Szabo *et al*., 2010).

Oyster mushrooms have been identified as a source of vitamin D since 1994, they are unique among non-animal foods for containing vitamin D. In mushrooms, ergosterol is the main sterol and precursor for vitamin D_2. When exposed to ultraviolet B (UVB) light (290-320 nm), ergosterol absorbs the light energy, leading to the isomerization of the molecule and cleavage of the bond between carbons 9 and 10, producing an unstable intermediate known as "pre- vitamin D_2." This pre-vitamin D_2 is then converted into vitamin D_2 through a thermally catalyzed process (Chen and Huang, 2010).

A study on enhancing vitamin D levels in healthy young adults found that consuming UV-B treated mushrooms, which are enriched with vitamin D, had a positive impact comparable to ingesting 28,000 IU of vitamin D_2 once a week for four weeks. The study observed a significant increase in serum 25(OH)D levels following the consumption of UV-B treated mushrooms. This increase was noted after the first dose and continued with subsequent doses, showing greater improvements compared to the placebo group. These findings demonstrate that UV-treated mushrooms effectively boost vitamin D status in individuals (Urbain *et al*., 2011).

Kristensen *et al.* (2012) conducted a study which showed the effect of UV-B rays on the vitamin D_2 content of the mushrooms during growth period. In the experiment the mushrooms were exposed to UV-B rays just prior to the harvesting phase. The results revealed that on the basis of fresh samples the vitamin D content was observed at 0.2-164 µg/100 g whereas exposing to the 1000 mJ/cm dose of UV rays showed the direct relationship with vitamin D_2 content of mushrooms. The results also revealed the inverse relationship between growth rate and vitamin D_2 content of mushrooms. At the same, the exposure to UV rays has no effect on the growth rate of mushrooms. UV-B exposure concluded the higher production of vitamin D_2 content with 32 µg/100 g in mushrooms at post-harvest level as compared during growth period which was observed at 24 µg/100 g. Other than this, the content of vitamin D_2 ranged from 0.2-1.5 µg/100 g in cultivated or wildly grown mushrooms having with or without exposure to sun rays. So, the results showed exposure to the UV light as compared to the sun rays have the improved effect on the vitamin D_2 content of mushrooms.

Phillips and Rasor (2013), studied that exposure of sliced white button mushrooms to sunlight is an easy, proved and trustworthy method which is also customer friendly in nature to enhance the vitamin D content of mushrooms as well it helps to improve the vitamin D status of people. In mushrooms vitamin D_2 content can increase from 25 to 100 per cent of the DRI (150 IU- > 600 IU) with sunlight exposure for 15 minutes either in clear or partially cloudy day period.

Edward *et al*. (2015) investigated the impact of UV-A (365 nm) and UV-C (254 nm) light exposure on the vitamin D_2 content of post-harvest oyster mushrooms (*Pleurotus ostreatus*). The study found a measurable increase in vitamin D_2 levels, indicating that both the wavelength and duration of UV light exposure significantly affect vitamin D_2 concentration. Additionally, consumer quality assessments revealed that mushrooms exposed to UV-C light were chewier compared to those exposed to UV-A light.

Nolle *et al.* (2017) reported the effect of direct sunlight during natural and solar drying in a forced convection type solar dryer on vitamin D_2 content of brown button mushrooms (*Agaricus bisporus*). The locally manufactured low-cost solar dryers considered as the promising substitute to the artificial UV-light to enhance vitamin D_2 content naturally in mushrooms followed by the development of safe vitamin D rich dried products.

Kalaras *et al*. (2012) investigated the effect of pulsed UV radiation on *A. bisporus* samples, applying 12 pulses to achieve a maximum vitamin D_2 concentration of about 27 µg/g dry weight (d.w.). In contrast, 60 pulses resulted in a significantly higher concentration of 124 µg/g d.w. Notably, vitamin D_2 levels in untreated mushroom samples were undetectable.

Krings and Berger (2014) conducted a study where the fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) were irradiated by UV-B rays with intensity of 11.5 W/m^2 and 310-320 nm wavelengths at 20ºC temperature for 1 hour duration. There were the changes observed in the concentrations of the photo-isomers lumisterol, tachysterol and pre vitamin D_2 along with positive effect was seen in the sterol and fatty acid concentration.

The highest increase of 22-Dihydroergocalciferol (vitamin D_4) was observed in the present study.

Slawinska *et al*., (2016) conducted an experiment to assess the stability of vitamin D_2 in dehydrated mushrooms during storage. The study focused on *Agaricus bisporus, Pleurotus ostreatus* and *Lentinula edodes* evaluating the impact of UV-B exposure on vitamin D_2 production. The findings revealed that, after a storage period of one and a half years, *Agaricus bisporus* retained 48.32 per cent of its initial vitamin D_2 content, while *Pleurotus ostreatus* and *Lentinula edodes* retained 66.90 per cent and 68.40 per cent, respectively. The study concluded that UV-B radiation positively affects vitamin D_2 content in mushrooms. Among the drying methods, freeze-dried button mushrooms exhibited higher vitamin D_2 levels compared to those dried in a hot air oven. However, it was noted that despite the initial increase in vitamin D_2 content due to UV exposure, there was a loss of vitamin D_2 during the storage period following dehydration.

Huang *et al*., (2015) conducted a study to investigate the effects of UV-B radiation on vitamin D_2 content and antioxidant properties in various mushroom fruiting bodies and mycelia. Eleven fresh mushroom varieties were exposed to UV-B rays for two hours. The study found a significant increase in vitamin D_2 levels in both the fruiting bodies and mycelia of the mushrooms. While there was a slight improvement in chelating and reducing activities, a decrease in scavenging activity was observed.

This study evaluated the effects of UV-C irradiation on vitamin D_2 formation and its bioavailability in rats. Fresh button mushrooms were exposed to UV-C irradiation at mean intensities of 0.403, 0.316, and 0.256 mW/cm² from distances of 30, 40, and 50 cm, respectively, for periods ranging from 2.5 to 60 minutes. Exposure to UV-C irradiation at 0.403 mW/cm² from a distance of 30 cm resulted in a time-dependent increase in vitamin D_2 concentrations, significantly higher than those produced at the lower intensities of 0.316 and 0.256 mW/cm² from distances of 40 and 50 cm, respectively. Additionally, the concentrations of vitamin D_2 produced after exposure to UV-C doses of 0.125 and 0.25 J/cm² for 2.5, 5, and 10 minutes were 6.6, 15.6, and 23.1 µg/g (Koyyalamudi *et al*., 2009).

Koyyalamudi *et al*., (2011) found that the amount of vitamin D_2 produced with pulsed UV light increased in direct proportion to the number of pulses applied. For instance, 9 pulses resulted in 161 µg/g dry weight (d.w.) of vitamin D_2. This is higher compared to other studies, such as one that reported 141 µg/g d.w. using continuous UV-C light at an intensity of 0.25 J/cm², applied for 10 minutes at a distance of 30 cm.

This study was conducted to identify the effects of UV-B exposure at high concentration (0.5, 0.75, and 1.0 mW/cm²) with varied doses such as 0.5, 1.0, and 1.5 J/cm² for different post- harvesting durations on 1st and 4th days on the formation of vitamin D_2 in Portabella mushrooms. The vitamin D_2 in treated mushrooms during storage was also observed. The concentration of vitamin D_2 production was observed 3.83µg/g dry solids in 8 min at 0.5 J/ cm² dose and 1.0 mW/ cm² intensity whereas at 0.5 J/ cm² dose with 0.5 mW/ cm² intensity the concentration was found at 3.75 µg/g in dry solids of mushrooms in 18 min. There was no significant effect of post harvesting durations was observed in the formation of vitamin D_2. The constant loss of vitamin D_2 was observed during storage (Roberts *et al* 2008).

A study was conducted to optimize UV-B intensity (1.00-2.50 W/ cm²) and exposure time (20-120 min) for maximum ergosterol conversion to vitamin D_2. The optimization studies indicated that exposure with intensity (2.00 W/ cm²) for 60 min showed maximum vitamin D formation. The analysis of different nutritional parameters revealed no significant impact on total soluble proteins while a significant increase in total phenolic and total flavonoid contents was observed. On the contrary, a significant reduction was observed in riboflavin content of *P. florida.* Overall, *P. florida* exposure to UV-B is a sustainable approach to enhance the vitamin D content for the development of fortified mushroom products or dietary supplements (Kaur *et al*., 2023).

An effective method for producing vitamin D_2 enriched mushroom powder using UV-B radiation on postharvest Shiitake, King Oyster, and Portobello mushrooms. The highest vitamin D_2 content, 1000 IU per gram of dry matter, was achieved with a specific UV-B dosage distribution. This technique also increased vitamin D_2 levels in shiitake by-products suitable for animal feed. Comprehensive assessments ensured the safety and

nutritional quality of the enriched mushroom products. While the enriched shiitake powder didn't significantly enhance human serum vitamin D status, it did help maintain it during winter months. This research offers valuable insights into mushroom-based vitamin D supplementation (Hidalgo-Sanz *et al*., 2023).

The study examined the effectiveness of UV-C radiation in prolonging the shelf life of oyster mushrooms. Treating the mushrooms with 4.0 kJ m^{-2} UV-C radiation prior to packaging and storing them at 4°C for 15 days yielded promising results. UV-C treated mushrooms displayed reduced color changes, lower tissue electrolyte leakage, and decreased levels of MDA (Melon dialdehyde), while maintaining higher soluble solid content, soluble protein, CAT (Catalase), PAL (Phenylalanine ammonia lyase) activities, and overall quality compared to untreated mushrooms. Additionally, UV-C treatment demonstrated potential for surface decontamination. Overall, this research suggests that UV-C treatment holds promise for extending the shelf life of oyster mushrooms, benefiting both producers and consumers (Wang *et al*., 2017a).

Other studies have also shown that UV light can enhance vitamin D_2 levels in *A. bisporus*. For example, Teichmann *et al*., (2007) reported an increase from 0.07 to 10.14 µg/g dry weight (d.w.), while Jasinghe and Perera (2006) observed levels reaching 56.5 µg/g d.w. Guan *et al*. (2016) found vitamin D_2 concentrations ranging from 790 µg/100 g d.w. to 1340 µg/100 g d.w. The variation in vitamin D_2 levels across these studies can be attributed to differences in irradiation conditions.

2.5 Vitamin D_2 Stability in Vitamin-D Enhanced Edible Mushrooms during Storage

A limited number of studies have investigated the retention of vitamin D_2 in both fresh and dried mushroom fruiting bodies exposed to UV radiation and stored at refrigeration temperatures (2-4°C). Previous research has generally shown a decrease in vitamin D content over time after UV exposure, though some studies noted an initial increase in vitamin D_2 levels that subsequently declined. For instance, fresh white button mushrooms stored at 20°C exhibited a first-order kinetic decrease in vitamin D_2 levels over 14 days (Roberts *et al*., 2008). Similarly, vitamin D_2 levels in sliced button mushrooms

stored at 30°C dropped from 12 µg/g dry matter (DM) to 8 µg/g DM over 11 days (Kalaras *et al*., 2012).

Shiitake and oyster mushrooms showed a slight increase in vitamin D_2 content during the first 24 hours of storage at refrigeration temperatures, after which the levels began to decline over the following 10 days (Slawinska *et al*., 2017). In the same study, button mushrooms exhibited a gradual rise in vitamin D_2 content over six days, with a subsequent decrease observed on days 7 and 8. Conversely, other studies found no significant loss of vitamin D_2 when mushrooms were stored at refrigerated temperatures for 8 days (Koyyalamudi *et al*., 2009) or 14 days (Guan *et al*., 2016).

The findings from these studies suggest that vitamin D-enhanced mushrooms can retain nutritionally significant amounts of vitamin D_2 when stored at refrigeration temperatures for up to one week. For example, UV-B exposed button, shiitake, and oyster mushrooms that were hot air-dried and then stored in plastic containers under dry, dark conditions at 20°C showed notable retention of vitamin D_2 over eight months. However, a gradual decline in vitamin D_2 levels was observed after 8 to 18 months. Specifically, the vitamin D_2 concentration in hot air-dried button mushrooms decreased from 14.3 to 9.3 µg/g dry matter (DM) over eight months, with a further reduction to 6.9 µg/g DM by the end of 10 months (Slawinska *et al*., 2017).

Rajarathnam *et al*. (2003) conducted a study on the biochemical changes associated with browning in *Agaricus bisporus* and *Pleurotus florida* mushrooms. They found that as storage temperature increased from 0 to 25°C, phenol oxidase activity increased up to 15°C, followed by a decrease at 25°C in both mushroom species. They concluded that the loss of water content in fresh mushrooms was directly related to phenol oxidase activity, which could be correlated with the visible degree of browning in the mushrooms.

Cheng *et al*. (2020) investigated moisture migration in shiitake mushrooms (*Lentinula edodes*) during storage and its relationship to quality deterioration. They observed that prolonged storage caused a right shift in immobilized water and a left shift in free water, leading to the merging of these two peaks by the end of storage. An increase

in the peak area of water in the cytoplasm and a decrease in the peak area of water in the vacuole indicated moisture migration from the vacuole to the cytoplasm. Additionally, this moisture migration was likely related to weight loss and the softening of the mushroom's texture.

During storage, various changes can occur, such as browning and fat oxidation. Murr and Morris (1975) investigated the effect of storage temperature on post-harvest changes in mushrooms. They suggested that post-harvest maturation was supported by the utilization of low molecular weight nitrogenous compounds formed through increased protein degradation. Additionally, they noted that increased discoloration during storage appeared to be correlated with a decrease in total phenols and an increase in o-diphenol oxidase (o-DPO) activity. Rai and Saxena (1989) observed a decrease in protein content with extended storage duration.

Aadhilakshmi (2022) investigated the impact of various chemical preservatives on the shelf life of pink oyster mushrooms. They found that 1 per cent acetic acid extended the shelf life of mushrooms for 6 days, whereas 0.2 per cent potassium metabisulphite extended the shelf life up to 12 days with less quality deterioration than other treatments. The application of KMS in combination with high-density polyethylene (HDPE) and modified atmospheric packaging (MOP) extended the shelf life to 16 days. Hence, the study concludes that KMS (0.2 per cent) + HDPE + MOP is the optimal packaging solution, ensuring the longevity and quality of pink oyster mushrooms through a well-rounded preservation strategy.

An exhaustive review of literature indicated that enrichment of vitamin D_2 in different types of mushrooms upon exposure to UV radiation and its retention during storage. Different types of UV radiation *viz.*, UV-A (315-400 nm), UV-B (290-315 nm), UV-C (190-290 nm) showed positive results in different types of mushrooms, but there is less literature in case of oyster mushroom (*Pleurotus eous*). Hence, the present study has scope to provide substantial evidence.

MATERIAL AND METHODS

III MATERIAL AND METHODS

This chapter describes the material used and methodology adopted for treating mushroom with UV radiation and storage of oyster mushroom. The study entitled "Studies on enhancement of vitamin D_2 in oyster mushroom (*Pleurotus eous*) using UV radiation" was conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore, during 2023-2024.

3.1 Source of *Pleurotus eous*

Pink oyster mushroom (*Pleurotus eous*) variety was cultivated in mushroom lab, GKVK, Bangalore and used in the present study. The procedure for oyster mushroom cultivation involves the following steps

1. Preparation of spawn
2. Substrate preparation
3. Spawning of substrate
4. Harvesting of mushroom

3.1.1 Preparation of spawn

Pure culture of *Pleurotus eous* was obtained from Advance centre of Skill development in Mushroom production Technology, Department of Agricultural Microbiology, UAS, Bangalore and it was maintained on Potato Dextrose Agar plate at 4 °C (Yohannes *et al*., 2020) and was sub cultured every four weeks using Potato dextrose agar medium (Jaspreet kaur, 2017).

Fully grown culture of *P. eous* was used for spawn production, spawn is a mushroom seed, used to propagate the mushroom mycelia in the desired solid substrate during cultivation. One kg of healthy sorghum grains was washed thoroughly in tap water and boiled with 1.5 L of water for 30 min until they became soft. The grains were spread on a sterile surface to drain out the excess of water. To the drained grains, 1.5-2 per cent of calcium carbonate was mixed to make the grains free from clumps. Two hundred grams of

grains were taken in polypropylene bags (18 cm × 12 cm) plugged with cotton and were sterilized at 121 °C for 20 min. After cooling at room temperature, the mycelium from a *Pleurotus eous* culture is placed onto sterile grain and incubated for 12-15 days at 24 ± 1 °C for mycelial growth (Raman *et al*., 2021).

3.1.2 Substrate preparation

Paddy straw was used as a substrate for cultivation (Telang *et al*., 2010). Paddy straw was procured from integrated farming system, University of Agricultural Sciences, GKVK, Bangalore. Paddy straw was chopped into small pieces (5 to 10 cm using chopper) for mushroom cultivation. Paddy straw was soaked in a plastic tub with water overnight (8 hours) and drained, resulting in a moisture content of approximately 70 per cent. Substrate was autoclaved at 15 psi, 121 °C for 90 min.

3.1.3 Spawning of substrate

The sterilized substrate was inoculated with grain spawn in a transparent polythene cover (60 × 30 cm) of 80 gauge thickness at a rate of 10 per cent (w/w) i.e.100 g of spawn / 1kg of substrate. Seed spawn was mixed with substrate layer wise, the mouth of the bags were closed with cotton plug and tied with rubber band and the bag was transferred to the incubation room for spawn run. The pin holes were also made in the bags manually for exchange of gases. The bags were incubated at 20-25º C under dark condition for 18 to 20 days. After colonization, the plastic bag was removed from the substrate and was placed in the growing room. These bags were watered (sprayed) twice a day to maintain humidity as well as moisture (Kumari and Achal, 2008).

3.1.4 Harvesting of mushroom

The fruit bodies were harvested after 21-25 days of incubation (Plate 1). Oyster mushroom was harvested by grasping each mushroom stalk individually and twisting the mushroom until it is pulled out of the substrate (Yohannes *et al*., 2020).

3.2 Treating of *Pleurotus eous* with UV radiation

Oyster mushrooms are used in three different forms (whole mushroom, sliced mushroom and powdered mushroom), separately for each experiment. Three different forms of oyster mushroom was exposed separately to UV radiation at different exposure time.

3.2.1 Treatment details

Treatment details			
Treatments	**UV radiation exposure time (minutes)**		
	Whole	Slice	Powder
T_1	0	0	0
T_2	10	10	10
T_3	20	20	20
T_4	30	30	30
T_5	40	40	40
T_6	50	50	50
T_7	60	60	60
T_8	70	70	70

3.2.2 Sample preparation

Mushrooms were harvested and sample preparation was started immediately after harvest. The mushrooms were cleaned and fresh healthy mushroom free from disease and colour change were used for the experiment. 100 g of oyster mushroom in three forms *viz*., whole (fresh), a sliced (chopped into pieces of 1 cm^2) and powdered (Hot air drying-Heating to a temperature of 60 °C along with air convection, the hot wind takes away the moisture inside the mushroom, thus achieving the purpose of drying, these samples was taken to the experimentation (Plate 2) (Nolle *et al*., 2017).

3.2.3 Imposition of treatment

The three forms of mushrooms were subjected to UV-C radiation (254 nm) for a duration of 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes and 70 minutes in laminar air flow (Plate 3). After treatment 100 g of mushrooms were packed in polypropylene bag. Then the packets were labelled and stored in refrigerator (Cardoso *et al*., 2021).

3.3 Observation

The physical properties (physiological weight loss), biochemical properties (vitamin D_2, ergosterol, protein, carbohydrates, minerals,), microbiological analysis (bacteria, yeast and mold) and sensory evaluation (for colour, texture) were carried out.

3.3.1 Procedures followed for taking the observations

The observations pertaining to the physical properties such as physiological weight loss; biochemical properties namely vitamin D_2, ergosterol, protein, carbohydrates, minerals and count of bacteria, yeast and mold were analysed. The sensory evaluation was carried out immediately after treatment. The procedures followed in taking all those observations are detailed below.

3.3.1.1 Physical properties

3.3.1.1.1 Physiological weight loss

The physiological weight loss (PWL) of the mushroom was determined by weighing the entire mushroom before and after storage using a digital electronic balance DS 450 (Essae Teraoka Ltd., India) relative to the initial weight (Shao *et al*., 2021). The results were analyzed and expressed as per cent weight loss using the following formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initital weight}} \times 100$$

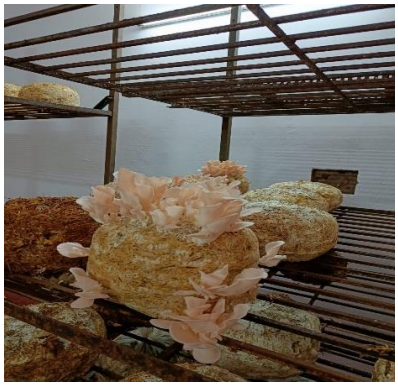
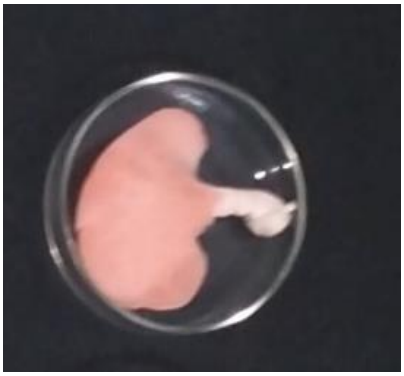
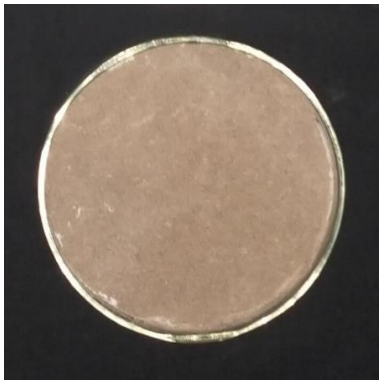




Plate 1. *Pleurotus eous* crop



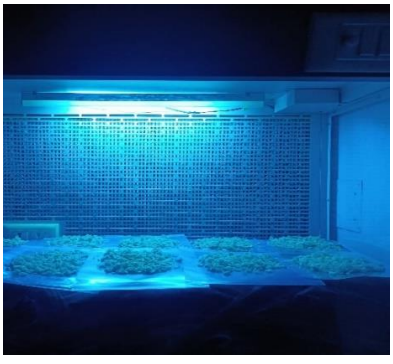




(a) Whole mushroom **(b) Sliced mushroom** **(c) Powdered mushroom**

Plate 2. Different forms of mushroom







(a) Whole mushroom exposed to UV radiation **(b) Sliced mushroom exposed to UV radiation** **(c) Powdered mushroom exposed to UV radiation**

Plate 3. Treatment imposition for different forms of mushroom

3.3.1.2 Biochemical analysis

3.3.1.2.1 Vitamin D_2 (HPLC method)

The working standard of vitamin D_2 (HPLC grade, Sigma-Aldrich) was prepared by dissolving 5 mg vitamin D_2 in 100 mL of distilled water (50 ppm). The different concentrations of vitamin D_2 (10, 20, 30, 40 and 50 ppm) was prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1.0 mL of working standard solution into a series of HPLC vials respectively. The volume was made upto 1 mL for all the concentrations. Further, these known concentrations were injected into the HPLC system (Shimadzu, Japan) equipped with UV detector and eluted through a reverse phase C18 column using acetonitrile / methanol (75:25) as the mobile phase at a flow rate of 2.3 mL /min and vitamin D_2 content was detected at 282 nm (Jashinge and Perera, 2006).

Mushroom sample (0.5g) was taken in 250 mL round bottom flask and mixed with 4 mL of sodium ascorbate solution, 50 mL of ethanol (95 per cent pure), and 10 mL of 50 per cent potassium hydroxide. Then the mixture was saponified for 1 hour under reflux at 80 °C. The mixture was cooled down and transferred into separating funnel. It was extracted first with 15 mL water, followed by 15 mL ethanol and then a three-stage n-pentane of volumes 50, 50 and 20 mL, respectively. The pooled organic layers were washed three times with 50 mL of 3 per cent potassium hydroxide in 5 per cent ethanol and then finally with deionized water until it is neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 40 °C and immediately redissolved in 5 mL ethanol. The samples were filtered through 0.45 µm filter units. A volume of 20 µl filtered sample was injected into the HPLC system and conditions were maintained similar to the standard. Quantification of vitamin D_2 was done using a standard curve and identification was achieved by comparison of retention times of sample and standard (Jashinge and Perera, 2006).

3.3.1.2.2 Ergosterol (HPLC method)

The working standard of ergosterol (HPLC grade, TCA chemicals) was prepared by dissolving 100 mg ergosterol in 100 mL of distilled water (1000 ppm). The different concentrations of ergosterol (200, 400, 600, 800 and 1000 ppm) was prepared by pipetting

out 0.2, 0.4, 0.6, 0.8 and 1.0 mL of working standard solution into a series of HPLC vials respectively. The volume was made upto 1 mL for all the concentrations. Further, these known concentrations were injected into the HPLC system (Shimadzu, Japan) equipped with UV detector and eluted through a reverse phase C18 column using acetonitrile / methanol (75:25) as the mobile phase at a flow rate of 2.3 mL/min and ergosterol content was detected at 282 nm (Jashinge and Perera, 2006).

Mushroom sample (0.5g) was taken in 250 mL round bottom flask and mixed with 4 mL of sodium ascorbate solution, 50 mL of ethanol (95 per cent pure), and 10 mL of 50 per cent potassium hydroxide. Then the mixture was saponified for 1 hour under reflux at 80 ℃. The mixture was cooled down and transferred into separating funnel. It was extracted first with 15 mL water, followed by 15 mL ethanol and then a three-stage n-pentane of volumes 50, 50 and 20 mL, respectively. The pooled organic layers were washed three times with 50 mL of 3 per cent potassium hydroxide in 5 per cent ethanol and then finally with deionized water until it is neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 40 ℃ and immediately redissolved in 5 mL ethanol. The samples were filtered through 0.45 µm filter units. A volume of 20 µl filtered sample was injected into the HPLC system and conditions were maintained similar to the standard. Quantification of ergosterol was done using a standard curve and identification was achieved by comparison of retention times of sample and standard. (Jashinge and Perera, 2006).

3.3.1.2.3 Protein

The protein estimation was done by Lowry's method. Reagents (Solution A-2 per cent Sodium carbonate in 0.1N NaOH; Solution B-0.5 per cent copper sulphate in 1 % sodium potassium tartarate; Solution C-Mix 50 ml of solution A with 1 mL of solution B just prior to use; Folin-Ciocalteu reagent; stock standard solution: 50 mg of bovine serum albumin in 50 mL of water; working standard solution: dilute 10 mL of the stock solution to 50 mL with water to obtain 200 µg protein/mL) was prepared. Protein was extracted from the sample by grinding 0.5 g of the sample with phosphate buffer in a pestle and mortar. Centrifuged and the supernatant was used for the protein estimation (Thimmaiah, 1999).

Estimation of protein

The working standard solution of 0.2, 0.4, 0.6, 0.8 and 1.0 mL was pippeted out into a series of test tube. The sample extract of 0.1 mL and 0.2 mL was pippeted out into two other test tubes. Volume was made upto 1mL in all the test tube. A tube with 1ml serves as the blank. Solution C of 5 mL was added, mixed well and incubated at room temperature for 10 min. FCR of 0.5 mL was added mixed well immediately incubated at room temperature in dark for 30 min. Absorbance was read at 660 nm against the blank. Standard graph was drawn and the protein content in the sample was calculated.

$$\text{Protein (\%)} = \frac{\text{value from graph} \times \text{total volume of extract}}{\text{aliquot volume} \times \text{weight of the sample taken for extract}} \times 100$$

3.3.1.2.4 Carbohydrates (Anthrone reagent method)

Hundred milligram of grinded mushroom was taken into a boiling tube. Hydrolysed by keeping in a boiling water bath for three hours with 5 mL of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceased. Volume was made up to 100 mL and samples will be centrifuged at 4000 rpm for 5 minutes, supernatant will be collected and evaporated by placing in water bath at 80 °C. The dried sample will be made up to 10 mL by dissolving the sugars. The standards will be prepared by taking 0.0, 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard and made upto 1 mL with distilled water. 0.5 mL of each sample will be taken into test tube made upto 1 mL with distilled water. 4 mL of anthrone reagent was added to each tube and mixed well. The test tubes were placed in boiling water bath for 8 minutes, then tubes were cooled. The absorbance was recorded at 630 nm (Thimmaiah, 1999).

$$\text{Carbohydrates (\%)} = \frac{\text{value from graph} \times \text{total volume of extract}}{\text{aliquot volume} \times \text{weight of the sample taken for extract}} \times 100$$

3.3.1.2.5 Minerals

Exact 0.25 g of oven dried mushroom samples were taken. 10 mL of tri-acid (HNO_3: H_2SO_4: Per-chloric acid, in the ratio 9:2:1, v/v) was added to sample and digested under the heater at 120 ℃ in fume-hood until the volatile content disappears retaining inorganic minerals. Carefully the flask were taken out of the heater and cooled to room

temperature. The extracted mineral contents volume was made up to 100 mL in volumetric flask and sample was filtered with Whatman No. 1 filter paper. This extract was stored until taken as aliquot for the further mineral estimations (Karosiya *et al*., 2019).

3.3.1.2.5.1 Atomic absorption spectrometer (AAS) study

AAS works based on absorption phenomena where metal ions are raised to the energy level sufficiently high to emit its characteristics radiation. These atom returns to the ground state at which they absorb the light energy characteristic to their own resonance wavelength. The light radiation emitting from hallow cathode lamp absorbed by the ground state atom will be proportional number of atoms in the flame and is determined as the amount of absorbed atom is directly proportional to its concentration. The instrument was integrated with the calibration with standards. Standard minerals taken were $CuSO_4$ (Copper), $ZnSO_4$ (Zinc), $FeSO_4$ (Iron) and $MnSO_4$ (Manganese). Standards were made according to the Table 2. Acetylene gas was used as ignition gas for the atomic excitation. 20 µl tri-acid digested sample were used for estimation of the mineral. The standard integrated calibration was done by putting on the compatible cathode lamp for estimation and estimation was done. For every analysis blank was used to make auto zero. The analysis was recorded by integrated software and data was downloaded (Karosiya *et al*., 2019).

Table 2: Mineral nutrient (mg/l) standards used in standard integrated AAS

Standards/Elements	Zn	Cu	Fe	Mn
Standard-1	0.5	1.0	2.0	1.0
Standard-2	1.0	2.0	4.0	2.0
Standard-3	1.5	3.0	6.0	3.0
Standard-4	2.0	4.0	8.0	4.0
Standard-5	2.5	5.0	10.0	5.0

3.3.1.2.5.2 Flame photometric Analysis of Potassium

Acid digested mushroom sample was used for estimation of potassium by flame photometer. Dilution factors were calibrated according to the level detection. After putting on the flame photometer compressor pressure adjusted was 4.5 kg/cm^2, turned on the liquid petroleum gas (LPG) and ensured that the flame was blue and shoot free. Potassium set up number was set i.e. 20 to 100 ppm. Distilled water was aspirated and standard was calibrated by aspiring standard potassium (KCl) from ascending to descending order concentration 10, 8, 6, 4, 2 ppm one after another and was ended with aspirating with double distilled water. Later sample analysis was carried out; care was taken where double distilled water was aspirated after every sample loading, flame photometer reading (FMR) was noted and expressed in percentage on dry weight basis (Karosiya *et al*., 2019).

3.3.1.2.5.3 Determination of Phosphorus

The phosphorus content of the tri-acid digested grain and straw samples were determined by Vanadomolybdo phosphoric yellow colour method in HNO_3 acid system (Jackson, 1973) and expressed in percentage on dry weight basis.

3.3.1.3 Microbiological analysis

3.3.1.3.1 Bacteria, Yeast and mold

Mushroom samples were analysed for bacteria, yeast and mold by employing the dilution plate count method (Somasegaran and Hoben, 1985). Total plate count and fungi count were determined as described by Atanda and Akano (1997). For enumeration of bacteria, yeast and mold, 1 g of sample was weighed aseptically and was diluted in 10 mL of distilled water and subsequent dilution were prepared upto 10^{-4} by transferring 1 mL of aliquot from 10^{-1} dilution to 9 mL of distilled water. The filterates serially diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and used for enumerating bacteria (10^{-4} dilution), yeast and mold (10^{-2} dilution) by plating on suitable agar medium in duplicates. Culture media used were plate count agar (PCA) for bacteria and potato dextrose agar (PDA) for yeast and mold. Required dilution of 1 mL was transferred to sterilized petriplates and 15-20 mL of respective media was poured into it. The plates were rotated in clockwise and anticlockwise

to attain uniform distribution of dilution with the culture media. The plates with PCA and PDA were allowed to solidify and incubated for 2-5 days at 26 ± 2 ºC. The plates were observed for bacteria, yeast/ mold. Total number of colonies was counted and results were expressed as colony forming units (cfu/g) and determined using the formula,

$$\text{Total count (cfu/g)} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{weight of sample (g)}}$$

3.3.1.4 Colour and texture

A sensory evaluation was done to assess the colour and texture of mushroom. The Sensory evaluation was conducted in the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore. The evaluation was done with 21 semi-trained panel members from the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore. The sensory evaluation for mushroom was done with 9 point hedonic scale with 21 panel members (Table 3.) (Lyn *et al*., 2020).

Table 3: Hedonic Scale (1-9) for sensory evaluation

Acceptability of quality parameters	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

The sensory evaluation in mushroom was done to identify which treatment had higher consumer preference. The products were scored for color and texture on a numerical scoring method (Amerine *et al*., 2013). The samples ranked for quality parameters in descending order of acceptability, 9-1.

3.4. The retention of Vitamin D_2 content in UV radiation induced oyster mushroom during storage

The experiment was conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore, during 2023-2024.

3.4.1. Sample preparation

By the observation of previous experiment, exposure duration of 50 minutes was selected which has a high vitamin D_2 in all the three forms of mushroom compared to different treatments and it was used in this study to investigate the retention of Vitamin D_2 content in UV radiation induced oyster mushroom during storage (Salemi *et al*., 2021; Slawinska *et al*., 2016).

3.4.2. Treatment details

Three treatments were used to study the retention of Vitamin D_2 content in UV radiation induced oyster mushroom during storage for two forms like whole and slice form *viz*., T_1 – raw mushroom stored at 4°C (Control), T_2 - UV induced mushroom stored at 4°C and T_3 - UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C.

Aadhilakshmi (2022) investigated the impact of various chemical preservatives on the shelf life of pink oyster mushrooms. They found that 1% acetic acid extended the shelf life of mushrooms for 6 days with less quality deterioration than other treatments.

Four treatments were used to study the retention of Vitamin D_2 content in UV radiation induced oyster mushroom during storage for powder form *viz*., T_1 - powdered mushroom stored at room temperature, T_2 - powdered mushroom stored at 4°C and T_3 - UV induced powdered mushroom stored at room temperature and T_4 - UV induced powdered mushroom stored at 4°C.

3.4.3. Imposition of treatment

The three forms of mushrooms were subjected to UV-C radiation (254 nm) for a duration of 50 minutes. Whole and sliced mushroom was treated with 1% acetic acid i.e., by dipping oyster mushrooms for 10 min in the 1% acetic acid and then placed on absorbent paper for 2 minutes under fan to remove excess surface liquid. After treatment 100 g of mushrooms were packed in polypropylene bag. Then the packets were labelled and stored in refrigerator at 4°C (Plate 4). Powdered mushroom was subjected to UV-C radiation for a period of 50 minutes and stored one set in 4°C and another at room temperature (Plate 5).

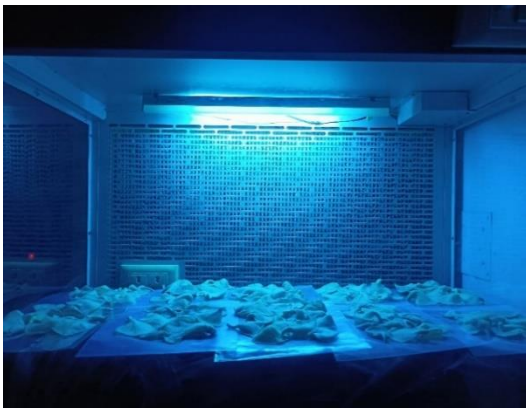
3.4.5 Observation

The physical properties (physiological weight loss), biochemical properties (vitamin D_2, ergosterol, protein, carbohydrates, minerals), microbiological analysis (for bacteria, yeast and mold) and sensory evaluation were carried out during storage period by employing standard procedures. The procedure for protein, carbohydrate from the book "Standard methods of biochemical analysis" authored by Thimmaiah (1999); physiological loss in weight by Shao *et al.* (2021); microbiological analysis by Atanda and Akano (1997); vitamin D_2, ergosterol analysis by Jashinge and Perera (2006); minerals analysis by Karosiya *et al*., (2019) were followed for analysis.

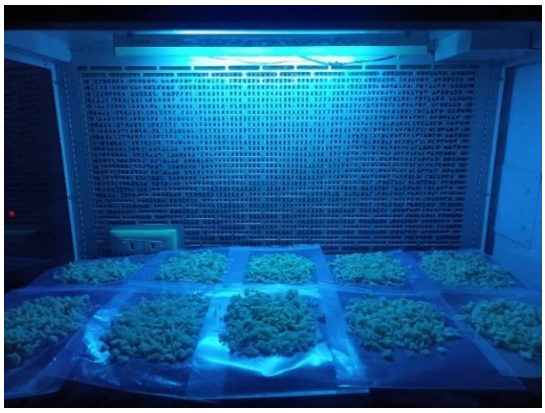
3.4.6 Sensory evaluation

The Sensory evaluation was conducted in the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore. The evaluation was done with 21 semi-trained panel members from the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore. The sensory evaluation for mushroom was done with 9 point hedonic scale with 21 panel members (Lyn *et al*., 2020). Hedonic scale suggested by Ranganna (1991) was followed. Details of the scale are given in Table 3.

The sensory evaluation in mushroom was done to identify which treatment would increase the shelf life of mushroom without affecting the consumer preference. The products were scored for appearance, color, aroma, texture and overall acceptability on a



(a) Whole mushroom exposed to UV radiation



(b) Sliced mushroom exposed to UV radiation



Whole mushroom dipped with 1% acetic acid

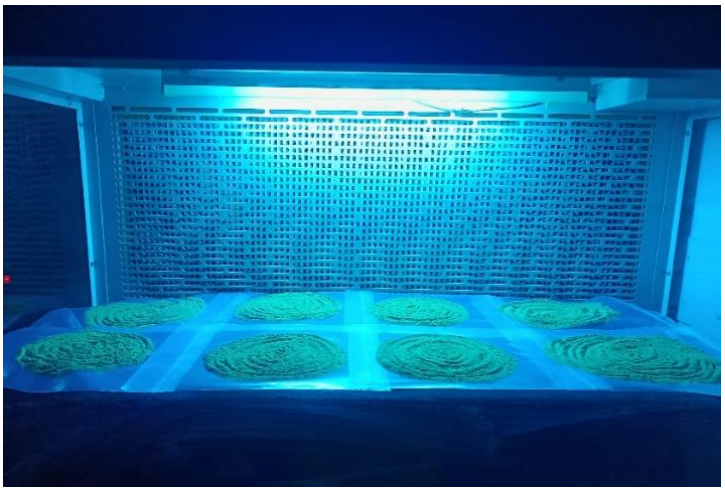
Stored at 4°C



Sliced mushroom dipped with 1% acetic acid

Stored at 4°C

Plate 4. Treatment imposition for whole and sliced mushroom



Powdered mushroom exposed to UV radiation



Stored at room temperature



Stored at 4°C

Plate 5. Treatment imposition for powdered mushroom

numerical scoring method (Amerine *et al*., 2013). The samples ranked for quality parameters in descending order of acceptability, 9-1.

3.5 Statistical analysis

The experiment was done in a completely randomized design (CRD) with 3 replications (Premakumar *et al*., 2020). The experiment data was subjected to analysis of variance (ANOVA) at 5 % level of significance by using opstat software (Singh *et al*., 2013).

RESULTS AND DISCUSSION

IV RESULTS AND DISCUSSION

The present chapter is to present and discuss the results obtained from the study entitled "Studies on enhancement of vitamin D_2 in oyster mushroom (*Pleurotus eous*) using UV radiation" was conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore, during 2023-2024.

4.1 Effect of UV radiation on Vitamin D_2 content in *Pleurotus eous*

The experiment was carried out to investigate the effect of UV radiation on vitamin D_2 content of mushroom. The results are described in detail and discussed below:

4.1.1 Effect of exposure time of UV irradiation on biochemical properties of three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

4.1.1.1 Vitamin D_2

The UV exposure of mushroom at different intervals of time leads to conversion of ergosterol to vitamin D_2, the results of conversion of ergosterol to vitamin D_2 are shown in Fig. 2a. There was significant enhancement of vitamin D_2 content in *Pleurotus eous* when exposed to UV rays for different time durations compared to T_1 (Control). When the whole mushroom was exposed to UV radiation, the maximum conversion of ergosterol to vitamin D_2 was observed in exposure duration of 50 minutes (T_6: 21.95±0.45 µg/g f.w. (fresh weight basis)) which was significantly higher than other treatments. Parallelly, in case of sliced mushroom significantly higher conversion of ergosterol to vitamin D_2 was observed when exposed to UV radiation for 50 minutes (T_6: 41.37±0.85 µg/g f.w.) compared to other treatments. Similarly, in powdered form also higher vitamin D_2 content was observed in exposure duration of 50 minutes (T_6: 246.31±5.12 µg/g d.w. (dry weight basis)) which was considerably higher than other treatments.

Higher conversion of ergosterol to vitamin D_2 was observed in all the three forms (whole, sliced and powdered) of mushroom exposed to UV radiation for 50 minutes (T_6) when compared to UV exposure times of 0 minutes(T_1), 10 minutes (T_2), 20 minutes (T_3), 30 minutes (T_4) and 40 minutes (T_5). This could be due to increase in duration of exposure

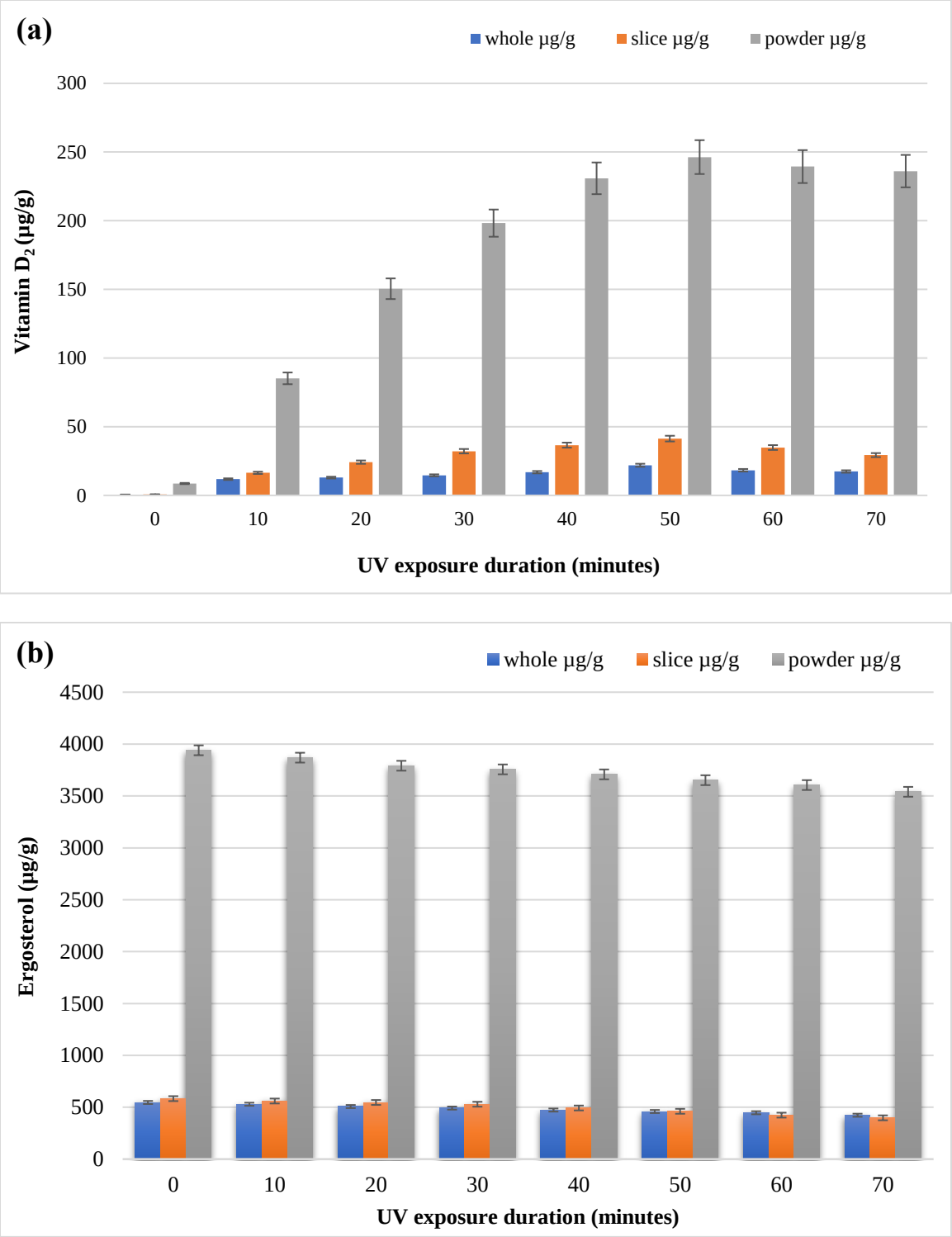


Fig. 2. Effect of exposure time of UV-C irradiation on (a) vitamin D_2 content (b) ergosterol content in three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

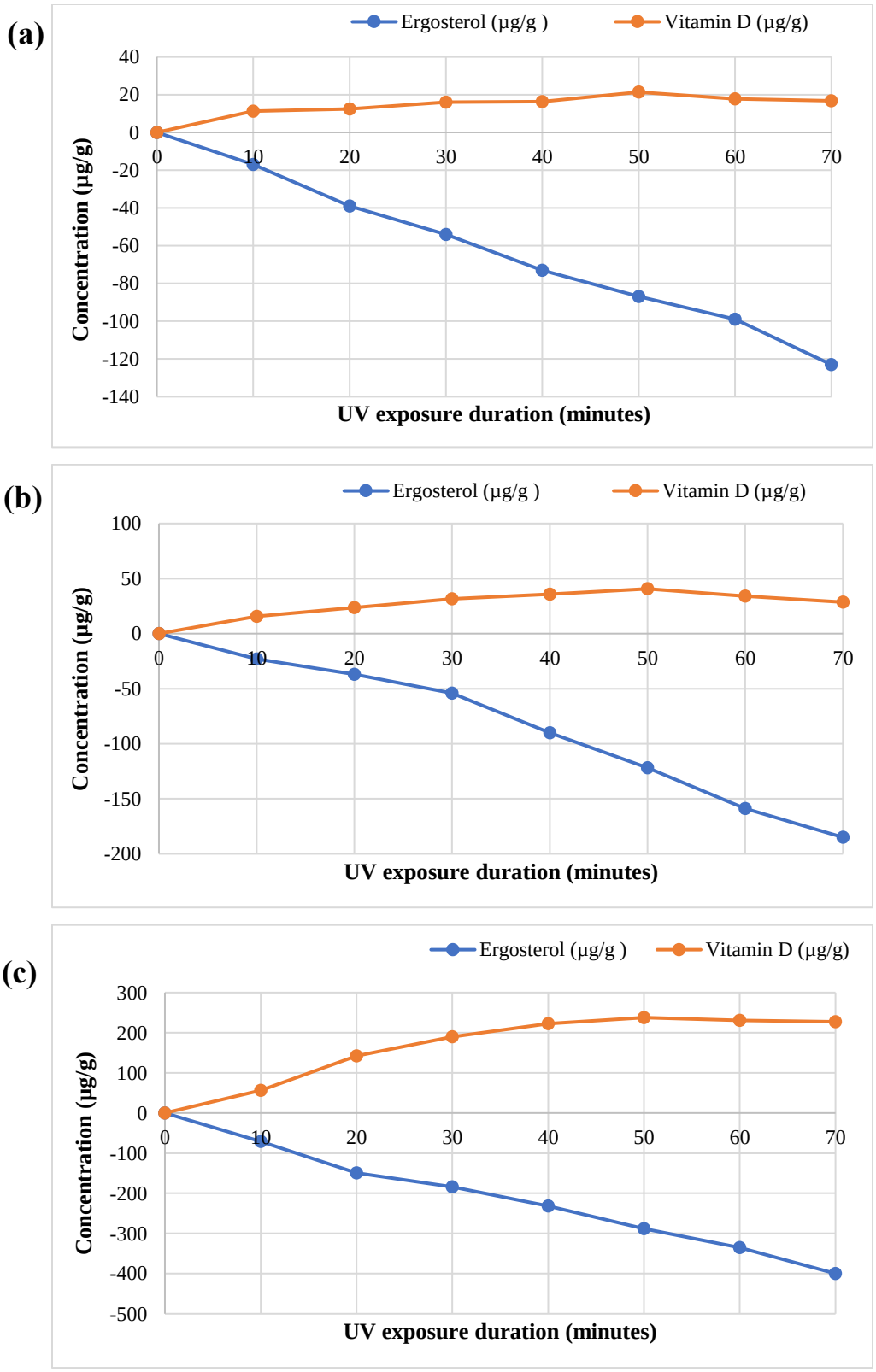


Fig. 3. Effect of exposure time of UV-C irradiation on both vitamin D_2 and ergosterol content in (a) whole (b) sliced and (c) powdered *Pleurotus eous* mushroom.

for UV leads to breakage of bond between C_9 and C_{10} in the B-ring of ergosterol to form pre-vitamin D_2, which undergoes thermally induced rearrangement to vitamin D_2 (Jasinghe and Perera, 2006).

4.1.1.2 Ergosterol

Ergosterol was the most abundant sterol found in mushrooms, which can be converted to vitamin D_2 upon exposure to UV radiation. The effect of UV-C irradiation time on ergosterol conversion in different forms of mushrooms are shown in Fig. 2b. The results indicated that, there was significant decrease in ergosterol content in all the three forms (Whole, sliced and powdered) of mushrooms with increase in the duration of exposure. In case of whole mushroom, the ergosterol content was decreased from 546 µg/g f.w. in T_1 (UV radiation exposure time of 0 minutes) to 423 µg/g f.w. in T_8 (UV radiation exposure time of 70 minutes). In case of sliced mushroom, the ergosterol content was decreased from 583 µg/g f.w. in T_1 (UV radiation exposure time of 0 minutes) to 398 µg/g f.w. in T_8 (UV radiation exposure time of 70 minutes). In case of powdered mushroom, the ergosterol content was decreased from 3940 µg/g d.w. in T_1 (UV radiation exposure time of 0 minutes) to 3540 µg/100g d.w. in T_8 (UV radiation exposure time of 70 minutes). This may be due to the exposure of mushroom surfaces to UV radiation caused a photochemical breakdown of the B-ring of ergosterol, leading to the production of pre-vitamin D_2, an intermediate compound. Subsequently, pre-vitamin D_2 was converted into vitamin D_2 through thermal isomerization (Jasinghe and Perera, 2006; Hu *et al*., 2020).

Upon exposure to a duration of 50 minutes, there was a decrease in ergosterol content by 87 µg/g f.w. led to an increase in vitamin D_2 content of 21.33 µg/g f.w. (for whole mushroom) (Fig. 3a). For sliced mushrooms, the ergosterol content dropped by 122 µg/g f.w., leading to an increase in vitamin D_2 content of 41.36 µg/g f.w. (Fig. 3b). In powdered mushrooms, the decrease in ergosterol was even more pronounced, with a reduction of 288 µg/g d.w. led to an increase in vitamin D_2 content of 246.31 µg/g d.w. (Fig. 3c).

Further exposing mushrooms to UV radiation from 50 to 70 minutes, a decrease in vitamin D_2 levels to 17.42±0.36 µg/g f.w. (whole), 29.34±0.60 µg/g f.w. (slice) and

236.10±4.91 µg/g d.w. (powder) was observed. This may be attributed to the either degradation of vitamin D_2 on over exposure for UV radiation or the formation of photo byproducts tachysterol and lumisterol from the intermediate compound pre-vitamin D_2 (Holick, 2012). The low conversion rate of ergosterol to vitamin D_2 could be the possibility of photo-degradation of vitamin D_2 on prolonged exposure to UV. Vitamin D_2 may also be subjected to the action of tissue mono-oxygenases that transform vitamin D_2 to 25 hydroxyvitamin D_2 and 25-dihydroxyvitamin D_2, thus reducing the overall conversion of ergosterol to Vitamin D_2 (Jasinghe and Perera, 2005).

Among three forms (Whole, sliced and powdered), highest vitamin D_2 content was observed in powder form, this may be due to the dried mushroom powder has a greater surface area exposed to UV light compared to whole and sliced mushrooms. This increased exposure area allows more effective conversion of ergosterol (the precursor to vitamin D_2) into vitamin D_2 (Hu *et al*., 2020). In the sliced mushrooms, conversion of ergosterol to vitamin D_2 was two times more than whole mushrooms. This could be due to sliced mushrooms offered more surface area to UV exposure resulting in higher vitamin D_2 formation compared to whole mushroom (Nolle *et al*., 2017).

4.1.1.3 Protein

The irradiation of mushroom samples for varied time period (0 – 70 minutes) showed no significant effect on protein content in all the three forms (Whole, sliced and powdered) (Table 4). The protein content of whole mushroom ranged between 2.77 per cent and 2.92 per cent f.w., sliced mushroom ranged between 2.89 per cent and 2.98 per cent f.w. and powdered mushroom ranged between 23.17 per cent and 25.38 per cent d.w. This could be due to primary, secondary, tertiary, and quaternary structures of proteins, which are stable under UV light because peptide bonds and the overall protein structure are not particularly susceptible to UV-induced photochemical reactions (Sanchez and Zervakis, 2001).The findings were consistent with those published by Simon *et al*. (2011) reporting that the irradiation of button mushroom with UV and sun light showed no significant effect on protein content as compared to untreated samples. Huang *et al*. (2015) reported that exposure of mushroom under UV radiations significantly enhanced the

Table 4. Effect of exposure time of UV-C irradiation on protein and carbohydrate content (%) in all three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

Treatments (UV radiation exposure time)	Protein (%)			Carbohydrate (%)		
	Whole (f.w.)	Slice (f.w.)	Powder (d.w.)	Whole (f.w.)	Slice (f.w.)	Powder (d.w.)
T_1- 0 minutes	2.86±0.05	2.95±0.08	25.38±1.65	3.45±0.06	3.80±0.02	32.60±0.55
T_2- 10 minutes	2.92±0.06	2.98±0.05	25.12±1.48	3.47±0.04	3.81±0.03	32.81±0.05
T_3- 20 minutes	2.85±0.14	2.97±0.07	24.32±0.09	3.48±0.03	3.80±0.01	31.48±2.00
T_4- 30 minutes	2.84±0.04	2.89±0.03	23.17±0.90	3.43±0.04	3.81±0.06	31.06±0.19
T_5- 40 minutes	2.77±0.05	2.94±0.05	24.33±0.61	3.43±0.03	3.83±0.01	30.82±0.19
T_6- 50 minutes	2.78±0.10	2.96±0.07	24.12±1.02	3.44±0.01	3.83±0.04	31.84±0.17
T_7- 60 minutes	2.80±0.10	2.95±0.03	24.47±0.51	3.47±0.07	3.82±0.07	30.62±0.55
T_8- 70 minutes	2.86±0.03	2.94±0.06	24.13±0.80	3.43±0.04	3.82±0.02	31.10±0.27
SE(m)	0.082	0.063	1.006	0.049	0.042	0.775
C.D.	N/A	N/A	N/A	N/A	N/A	N/A

vitamin D_2 content but no significant changes were found in other nutritional composition of mushroom. Moreover, Krings & Berger (2014) reported no change in amino acid content of UV irradiated *Pleurotus ostreatus* and control samples.

4.1.1.4 Carbohydrates

Carbohydrates, a key nutritional component in mushrooms, play an important role in their energy content and overall nutritional profile. In the present study, UV radiation showed no significant impact on carbohydrate levels across all three forms (Whole, sliced and powdered) of mushrooms (Table 4). The carbohydrate content of whole mushroom ranged between 3.43 per cent and 3.48 per cent f.w., while sliced mushroom ranged between 3.80 per cent and 3.83 per cent f.w. The powdered mushroom due to concentration from drying, had a notably higher carbohydrate content ranged between 30.62 per cent and 32.81 per cent d.w. This may be due to carbohydrates, including polysaccharides like cellulose and starch, are made up of sugar units linked by glycosidic bonds. These bonds are relatively stable and do not undergo significant photochemical reactions that would lead to their degradation when exposed to UV light (Zhu and Wang, 2020).

4.1.1.5 Minerals

Minerals are essential dietary components that contribute significantly to the nutritional value of mushrooms, offering health benefits such as supporting enzyme function and maintaining proper metabolism. In this study, the mineral content of mushrooms specifically phosphorus (P), potassium (K), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) was measured on a dry weight basis. The results indicated that there was no significant change in mineral content upon exposure to UV radiation.

Table 5 represents the mineral content of whole mushroom where phosphorus (P) ranged from 1.52 per cent to 1.64 per cent, while potassium (K) varies between 3.59 per cent to 3.62 per cent, iron (Fe) varied from 443.87 to 450.47 ppm, manganese (Mn) ranged between 33.27 and 34.80 ppm, copper (Cu) ranged from 31.00 to 32.80 ppm and zinc (Zn) ranged from 233.20 to 235.80 ppm.

Table 5. Effect of exposure time of UV-C irradiation on minerals in whole *Pleurotus eous* mushroom

Treatments (UV radiation exposure time)	P (%)	K (%)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
T_1- 0 minutes	1.53±0.05	3.61±0.01	449.67±2.33	34.53±0.17	32.33±0.24	235.80±2.08
T_2- 10 minutes	1.58±0.00	3.59±0.02	446.6±0.80	33.73±0.63	32.80±0.46	233.20±0.90
T_3- 20 minutes	1.62±0.01	3.61±0.01	450.47±1.54	34.80±0.30	32.47±0.35	234.33±1.92
T_4- 30 minutes	1.59±0.00	3.60±0.01	447.47±2.54	33.27±0.29	32.53±0.13	235.20±0.34
T_5- 40 minutes	1.52±0.01	3.62±0.00	443.87±2.35	33.00±0.23	31.00±0.41	233.00±0.87
T_6- 50 minutes	1.55±0.04	3.59±0.01	448.47±3.84	34.07±0.24	31.87±0.63	234.87±0.47
T_7- 60 minutes	1.53±0.02	3.60±0.00	447.80±1.94	34.40±0.30	31.67±0.17	234.53±0.17
T_8- 70 minutes	1.64±0.06	3.60±0.01	446.40±1.97	34.33±0.96	32.73±0.63	234.47±2.02
SE(m)	0.036	0.012	2.317	0.467	0.422	1.327
C.D.	N/A	N/A	N/A	N/A	N/A	N/A

Table 6. represents the mineral content of slice mushroom where phosphorus (P) ranged from 2.01 per cent to 2.09 per cent, while potassium (K) varies between 3.53 per cent to 3.58 per cent, iron (Fe) varied from 436.8 to 457.73 ppm, manganese (Mn) ranged between 28.23 and 30.07 ppm, copper (Cu) ranged from 33.53 to 35.53 ppm and zinc (Zn) ranged from 292 to 305.07 ppm.

Table 7. represents the mineral content of powdered mushroom where phosphorus (P) ranged from 2.11 per cent to 2.19 per cent, while potassium (K) varies between 3.51 per cent to 3.56 per cent, iron (Fe) varied from 436.33 to 469.67 ppm, manganese (Mn) ranged between 31.27 and 33.33 ppm, copper (Cu) ranged from 39.47 to 40.8 ppm and zinc (Zn) ranged from 240.2 to 245.87 ppm.

There was no significant change in mineral content upon UV exposure may be due to their stable chemical structures, lack of photochemical reactivity, and absence of UV-sensitive functional groups and also the energy provided by UV light is generally insufficient to break the strong ionic or covalent bonds in minerals (Rao, 2013).

The variation in mineral content among the three forms (Whole, sliced and powdered) of mushrooms, this may be attributed to differences in their cultivation such as, duration, substrate used and the growing conditions (Wiafe-Kwagyan *et al*., 2016).

4.1.2 Effect of exposure time of UV-C irradiation on physical properties of three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

4.1.2.1 Physiological weight loss

Mushrooms have high water content, making them susceptible to dehydration, especially when exposed to external factors like UV radiation. Fig. 4. represents weight loss of whole and slice mushroom upon exposure to UV radiation. The results indicated a significant increase in weight loss in all the treatments of whole and sliced mushrooms. For whole mushrooms, the weight loss was significantly increased with increase in duration of exposure in all the treated samples compared to control. The minimum weight loss was observed in the samples treated with UV for a duration of 10 minutes (T_2: 2.15 per cent). The maximum weight loss was occurred in sample exposed for a duration of 70 minutes

Table 6. Effect of exposure time of UV-C irradiation on minerals in sliced *Pleurotus eous* mushroom

Treatments (UV radiation exposure time)	P (%)	K (%)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
T_1- 0 minutes	2.04±0.02	3.56±0.01	438.67±21.30	30.07±0.29	35.13±0.17	297.93±3.01
T_2- 10 minutes	2.01±0.00	3.53±0.01	436.80±12.06	29.73±1.07	34.93±0.17	305.07±3.01
T_3- 20 minutes	2.03±0.03	3.54±0.00	440.60±12.43	29.80±0.72	35.53±0.59	296.53±0.46
T_4- 30 minutes	2.09±0.00	3.58±0.00	457.73±6.46	28.23±0.52	33.60±0.64	292.07±0.88
T_5- 40 minutes	2.05±0.04	3.56±0.01	441.67±2.40	29.73±0.24	34.13±0.24	298.33±6.23
T_6- 50 minutes	2.03±0.02	3.56±0.01	439.67±4.64	29.73±0.35	34.87±1.46	292.00±0.64
T_7- 60 minutes	2.09±0.02	3.53±0.02	437.13±4.05	28.40±0.60	34.73±0.29	298.40±5.81
T_8- 70 minutes	2.03±0.01	3.56±0.01	449.73±9.61	28.73±0.48	33.53±0.48	298.47±6.57
SE(m)	0.024	0.014	10.795	0.592	0.646	4.114
C.D.	N/A	N/A	N/A	N/A	N/A	N/A

Table 7. Effect of exposure time of UV-C irradiation on minerals in powdered *Pleurotus eous* mushroom

Treatments (UV radiation exposure time)	P (%)	K (%)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
T_1- 0 minutes	2.16±0.01	3.54±0.03	440.60±0.69	31.27±0.54	39.60±0.34	240.47±1.67
T_2- 10 minutes	2.19±0.01	3.57±0.00	452.27±4.55	31.87±0.24	40.87±0.17	242.87±0.75
T_3- 20 minutes	2.18±0.02	3.56±0.00	465.73±6.95	32.40±1.17	40.67±0.35	241.47±0.70
T_4- 30 minutes	2.13±0.01	3.56±0.01	463.07±3.34	31.73±0.65	40.00±0.41	245.87±1.01
T_5- 40 minutes	2.12±0.01	3.54±0.02	469.67±18.05	33.33±0.70	39.93±0.85	241.60±2.00
T_6- 50 minutes	2.13±0.03	3.52±0.01	458.67±22.20	32.93±0.35	39.47±0.63	242.93±0.86
T_7- 60 minutes	2.11±0.00	3.51±0.01	436.33±2.83	32.33±0.81	39.73±0.52	245.20±0.91
T_8- 70 minutes	2.17±0.01	3.54±0.02	450.87±9.73	32.27±0.40	40.73±0.24	244.13±3.47
SE(m)	0.017	0.018	11.196	0.672	0.488	1.679
C.D.	N/A	N/A	N/A	N/A	N/A	N/A

(T_8: 5.15 per cent), which was followed by samples treated with exposure duration of 60 minutes (T_7: 4.48 per cent), 50 minutes (T_6: 3.96 per cent), 40 minutes (T_5: 3.43 per cent), 30 minutes (T_4: 3.09 per cent) and 20 minutes (T_3: 2.47 per cent), however we could not find any weight loss in T_1. A similar trend was observed in sliced mushrooms, where the highest weight loss was recorded at an exposure duration of 70 minutes (T8: 7.18 per cent), compared to other treatments and lowest weight loss was found in untreated sample (T_1) and in the sample exposed to UV for 10 minutes (T_2: 2.67 per cent).

The maximum weight loss in treatment T_8 was attributed to the longer duration of exposure which leads to decrease in moisture content. This is because UV light can disrupt the integrity of cell walls, leading to increased permeability and subsequent water evaporation from the mushroom tissues, contributing to their weight loss. The weight reduction was greater in sliced mushroom compared to whole mushrooms. This may be due to larger exposure area of sliced mushroom resulting in more significant moisture loss (Cardwell *et al*., 2018).

4.1.2.2 Colour and texture

Color and texture are important sensory attributes of mushrooms, influencing consumer perception and quality. A visual quality assessment indicated no significant difference in color of mushrooms treated with UV radiation, while a slight alteration in texture was observed in case of whole and sliced mushroom. This textural change may result from UV-induced disruption of the cell wall, affecting the firmness of the mushrooms. UV light can penetrate the cell wall, potentially weakening its structure and leading to slight softening (Kalaras *et al*., 2012). There was no notable change in the color and texture of powdered mushrooms following UV treatment. This is because the powdering process itself modifies the mushroom's original appearance, reducing the potential for further noticeable alterations due to external factors like UV exposure (Table 8).

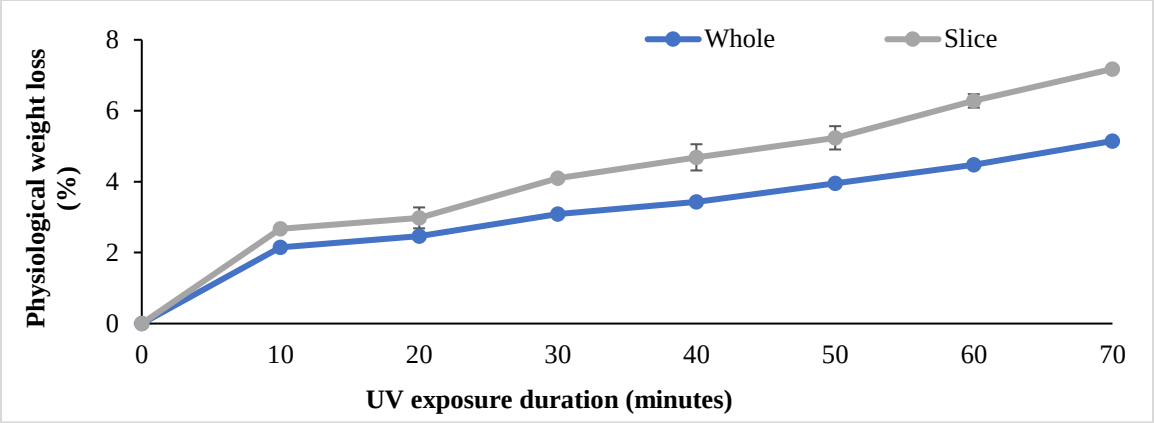


Fig. 4. Effect of exposure time of UV-C irradiation on physiological weight loss (%) in whole and sliced *Pleurotus eous* mushroom

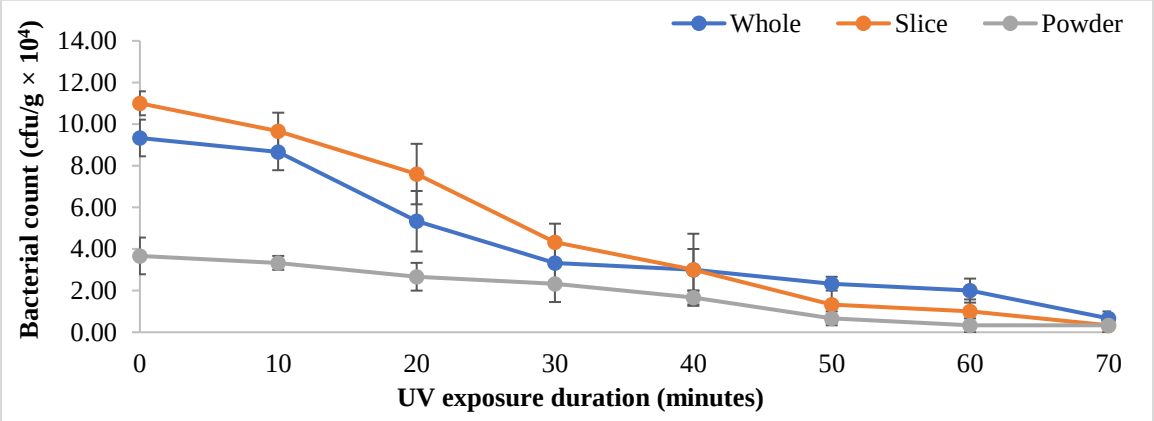


Fig. 5. Effect of exposure time of UV-C irradiation on bacterial population in all three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

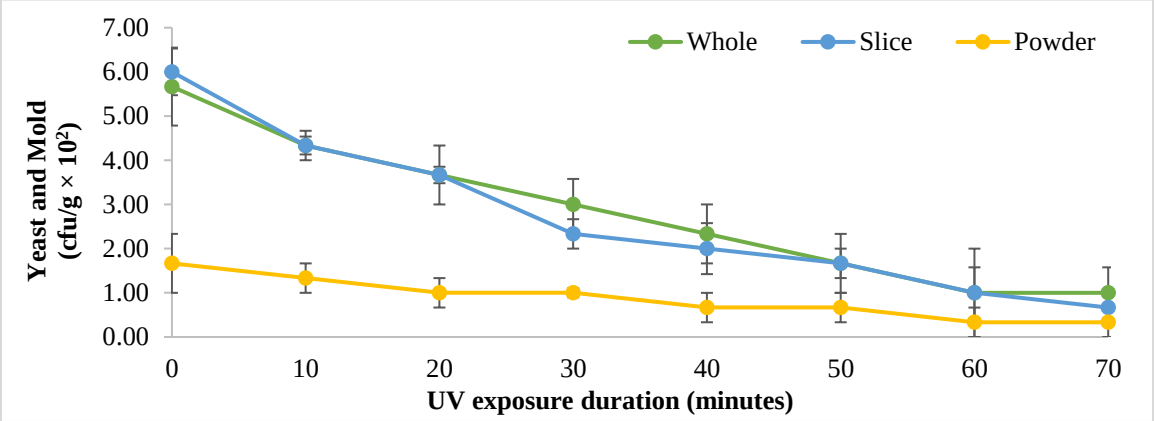


Fig. 6. Effect of exposure time of UV-C irradiation on yeast or mold population in all three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

Table 8. Sensory scores of colour and texture

Treatments (UV radiation exposure time)	Colour			Texture		
	Whole	Slice	Powder	Whole	Slice	Powder
T_1- 0 minutes	8.54	8.45	8.71	8.46	8.54	8.62
T_2- 10 minutes	8.52	8.43	8.69	8.44	8.52	8.60
T_3- 20 minutes	8.56	8.47	8.73	8.48	8.56	8.64
T_4- 30 minutes	8.44	8.35	8.61	8.36	8.44	8.52
T_5- 40 minutes	8.51	8.42	8.68	8.43	8.51	8.59
T_6- 50 minutes	8.40	8.31	8.56	8.32	8.40	8.48
T_7- 60 minutes	8.53	8.44	8.70	8.45	8.53	8.61
T_8- 70 minutes	8.34	8.25	8.50	8.26	8.34	8.42
SE(m)	0.094	0.091	0.097	0.093	0.092	0.097
C.D.	N/A	N/A	N/A	N/A	N/A	N/A

4.1.3 Effect of exposure time of UV-C irradiation on microbial population in all the three forms (Whole, sliced and powdered) of *Pleurotus eous* mushroom

4.1.3.1 Bacteria

The populations of bacteria significantly influence the quality of mushrooms after harvest. It was found that the bacterial count was significantly reduced from T_1 (Exposure duration of 0 minutes) to T_8 (Exposure duration of 70 minutes) upon exposure to UV radiation in all the three forms (Whole, sliced and powdered) of mushroom (Fig. 5). In case of whole mushroom, Initial bacterial count was 9.33×10^4 cfu/g (T_8). After UV exposure for 10 minutes viable count was reduced to 8.67×10^4 cfu/g (T_2), after 20 minutes the viable count was reduced to 5.33×10^4 cfu/g (T_3) and after 30 minutes reduced to 3.33×10^4 cfu/g (T_4). Further time increment showed only marginal decrease in bacterial population.

For sliced mushrooms, the initial bacterial count was 11.00×10^4 cfu/g (T_1). After 10 minutes of UV exposure, the viable count dropped to 9.67×10^4 cfu/g (T_2), and after 20 minutes, it further reduced to 7.63×10^4 cfu/g (T_3). By 30 minutes, the count significantly declined to 4.33×10^4 cfu/g (T_4). Beyond 30 minutes, increases in exposure time resulted in only a slight reduction in the bacterial population. For powdered mushrooms, the initial bacterial count was 3.67×10^4 cfu/g (T_1). After 10 minutes of UV exposure, the viable count dropped to 3.33×10^4 cfu/g (T_2), then further reduced to 2.67×10^4 cfu/g after 20 minutes (T_3), and to 2.33×10^4 cfu/g after 30 minutes (T_4). Increasing exposure time beyond this point only resulted in a marginal reduction in bacterial population.

The decrease in the population of bacteria with increase in the exposure time may be due to damage to nucleic acids upon UV exposure as the nucleotides interact with UV light. This light absorption elicits the formation of mutagenic DNA lesions including cyclo-butane pyrimidine dimers and 6 - 4 photoproducts. Both purines and pyrimidines can absorb UV radiation, although pyrimidines are thought to be highly photoreactive. When UV light breaks down nucleotides, DNA replication is halted, leading to cell death (Kim *et al*., 2017). There was no significant reduction in the bacterial population after 30 minutes of exposure to UV radiation, this could be due to 20 to 30 minutes of exposure time was adequate for disinfection or surface sterilization (Katara *et al*., 2008). According to the FSSAI, 2023 the aerobic microbial count should be within the limit of $1x10^4$ cfu/g to $1x10^5$ cfu/g, however in our study the bacterial population was within the limit in all the treatments.

4.1.3.2 Yeast and mold

The yeast and mold populations play a crucial role in determining the quality of mushrooms after they are harvested. Research indicates that exposure to UV radiation significantly decreases the yeast and mold count from T_1 (Exposure duration of 0 minutes) to T_8 (Exposure duration of 70 minutes) across all three types (Whole, sliced and powdered) of mushrooms (Fig. 6).

For whole mushrooms, the initial yeast and mold was measured at 5.67×10^2 cfu/g (T_1). After 10 minutes of UV exposure, the viable count declined to 4.33×10^2 cfu/g (T_2),

after 20 minutes, it decreased further to 3.67×10^2 cfu/g (T_3), and after 30 minutes (T_4), it reached 3.00×10^2 cfu/g. Subsequent increases in exposure time resulted in only a slight decrease in the yeast and mold population.

In the case of sliced mushrooms, the initial yeast and mold count was 6.00×10^2 cfu/g (T_1). Following 10 minutes of UV treatment, the viable count decreased to 4.33×10^2 cfu/g (T_2), after 20 minutes (T_3), it further declined to 3.67×10^2 cfu/g. After 30 minutes of exposure (T_4), the count had significantly reduced to 2.33×10^2 cfu/g. Beyond this duration, additional exposure time led to only marginal reductions in yeast and mold populations.

For powdered mushrooms, the initial yeast and mold count was recorded at 6.67×10^2 cfu/g (T_1). After 10 minutes of exposure to UV radiation, the viable count dropped to 1.33×10^2 cfu/g (T_2), after 20 minutes, it further decreased to 1.00×10^2 cfu/g (T_3), and by 30 minutes, it was at 1.00×10^2 cfu/g (T_4). Increasing the exposure time beyond 30 minutes resulted in non-significant reduction in yeast and mold count to 0.67×10^2 cfu/g (T_5 and T_6) and 0.33×10^2 cfu/g (T_7 and T_8).

The decrease in yeast and mold population with increasing exposure time could be due to UV radiation can significantly inhibit the growth by damaging their cellular DNA. This can lead to mutations, along with the development of pyrimidine and cyclobutyl-type dimers, inhibiting their reproduction and metabolic functions, which ultimately leads to decreased population (Watanabe *et al*., 2010) There was no significant reduction in the yeast and mold population after 30 minutes of exposure to UV radiation, this could be due to 20 to 30 minutes of exposure time was adequate for disinfection or surface sterilization (Katara *et al*., 2008). According to the FSSAI, 2023 the yeast and mold count should be within the limit of $1x10^2$ cfu/g to $1x10^4$ cfu/g, however in our study the yeast and mold count was within the limit in all the treatments.

This experiment concludes that UV exposure duration of 50 minutes (T_6) showed highest conversion of ergosterol to vitamin D_2 in all the three forms (Whole, sliced and powdered) with less or no significant reduction in quality and other nutritional

composition. Hence, UV exposure duration of 50 minutes (T_6) has been considered as a better duration of exposure and used further in storage study.

4.2 The retention of Vitamin D_2 content in UV radiation induced *Pleurotus eous* during storage

The experiment was carried out to determine the effect of UV radiation, acetic acid preservative (1%) and storage temperature on retention of Vitamin D_2 content in UV radiation induced *Pleurotus eous* during storage.

4.2.1 Effect of UV radiation and acetic acid preservative (1%) on biochemical properties of whole and sliced *Pleurotus eous* mushroom during storage

4.2.1.1 Vitamin D_2

The influence of UV radiation and acetic acid preservative (1%) on the vitamin D_2 content of *Pleurotus eous* (whole and sliced) during storage is presented in Table 9 and 10. Table 9 represents the vitamin D_2 content of whole mushroom during storage, the Initial vitamin D_2 content was recorded as 0.65±0.01 µg/g f.w. in T_1 (whole mushroom stored at 4°C (Control)), 19.84±0.41 µg/g f.w. in T_2 (whole mushroom exposed to UV for 50 minutes and stored at 4°C) and 19.73±0.49 µg/g f.w. in T_3 (whole mushroom exposed to UV for 50 minutes, treated with 1% acetic acid and stored at 4°C). Throughout the storage period, the vitamin D_2 content significantly decreased in all treatments, with T_3 exhibiting the highest retention of vitamin D_2 at 18.38 ± 0.24 µg/g f.w. after six days. This was followed by T_2, which had a retention of 14.85 ± 0.19 µg/g f.w., while T_1 showed the lowest retention with 0.43 ± 0.01 µg/g f.w.

The same trend was observed in case of sliced mushroom, where T_3 (sliced mushroom exposed to UV for 50 minutes, treated with 1% acetic acid and stored at 4°C) showed no significant reduction of vitamin D_2 content (41.45±0.54 µg/g f.w.), followed by T_2 (sliced mushroom exposed to UV for 50 minutes and stored at 4°C) (34.53±0.45 µg/g f.w.) and the highest reduction was observed in T_1 (sliced mushroom stored at 4°C : 0.46±0.01 µg/g f.w.) (Table 10).

Table 9. Effect of UV radiation and acetic acid preservative (1%) on change in vitamin D_2 content (µg/g) of whole *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	0.65 ± 0.01^{Ba}	0.56 ± 0.00^{Bab}	0.57 ± 0.01^{Cabc}	0.53 ± 0.00^{Cabc}	0.48 ± 0.00^{Cbc}	0.45 ± 0.01^{Cc}	0.43 ± 0.01^{Cc}
T_2	19.84 ± 0.41^{Aa}	18.84 ± 0.27^{Ab}	18.32 ± 0.24^{Bb}	17.38 ± 0.36^{Bc}	16.14 ± 0.24^{Bd}	15.53 ± 0.20^{Bde}	14.85 ± 0.19^{Be}
T_3	19.73 ± 0.49^{Aa}	19.57 ± 0.33^{Aa}	19.24 ± 0.25^{Aab}	19.04 ± 0.47^{Aab}	18.69 ± 0.31^{Aab}	18.67 ± 0.24^{Aab}	18.38 ± 0.24^{Ab}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 10. Effect of UV radiation and acetic acid preservative (1%) on change in vitamin D_2 content (µg/g) of sliced *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	0.65 ± 0.01^{Ba}	0.62 ± 0.01^{Bab}	0.58 ± 0.01^{Cbc}	0.56 ± 0.00^{Ccd}	0.53 ± 0.01^{Cde}	0.49 ± 0.01^{Cef}	0.46 ± 0.01^{Cf}
T_2	43.81 ± 0.91^{Aa}	42.62 ± 0.63^{Aab}	41.58 ± 0.54^{Bb}	39.16 ± 0.82^{Bc}	36.48 ± 0.54^{Bd}	35.24 ± 0.45^{Bd}	34.53 ± 0.45^{Bd}
T_3	43.47 ± 1.08^{Aa}	43.33 ± 0.73^{Aa}	43.15 ± 0.56^{Aa}	42.87 ± 1.06^{Aa}	42.53 ± 0.72^{Aa}	42.42 ± 0.55^{Aa}	41.45 ± 0.54^{Aa}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

The reduction in vitamin D_2 during storage may be due to cytochrome P450 enzymes present in oyster mushrooms, which are known to be involved in hydroxylating vitamin D_2 into 25-hydroxyvitamin. This enzymatic transition is a crucial step in the metabolic pathway of vitamin D, but it can also lead to a reduction in vitamin D levels during storage (Cardwell *et al*., 2023). The ability of acetic acid to modulate pH, act as a competitive inhibitor, exhibit antioxidant properties, and chelate metal ions contributes to its effectiveness in preventing enzymes involved in the degradation of vitamin D when used as a preservative, thus there was less degradation of vitamin D_2 was observed (Temova Rakusa *et al*., 2021).

4.2.1.2 Ergosterol

In the current investigation, storage had no significant effect on the mushroom's ergosterol concentration for whole and sliced mushrooms. For whole mushrooms, the initial ergosterol content was recorded as 0.52 mg/g in T_1 (whole mushroom stored at 4°C (Control)), 0.46 mg/g in T_2 (whole mushroom exposed to UV for 50 minutes and stored at 4°C) and 0.47 mg/g in T_3 (whole mushroom exposed to UV for 50 minutes, treated with 1% acetic acid and stored at 4°C) (Table 11). For sliced mushrooms, the initial ergosterol content was recorded as 0.56 mg/g in T_1 (sliced mushroom stored at 4°C (Control)), 0.45 mg/g in T_2 (sliced mushroom exposed to UV for 50 minutes and stored at 4°C) and 0.46 mg/g in T_3 (sliced mushroom exposed to UV for 50 minutes, treated with 1% acetic acid and stored at 4°C) (Table 12). Over the course of storage, the ergosterol content did not significantly alter in all the treatments of whole and sliced mushrooms. The absence of light that would trigger the conversion of ergosterol to vitamin D_2, further contributes to the stability of ergosterol during storage (Villares *et al*., 2014).

4.2.1.3 Protein

Protein is one of the nutritional and functional components in mushrooms and related to texture, flavour of oyster mushrooms which directly determined commercial value. The protein content of the mushroom during the course of storage were shown in Table 13 and 14. On a fresh weight basis, the initial protein content ranged from 3.42 to 3.46 per cent (For whole mushroom) and 3.84 to 3.89 per cent (for sliced mushroom). There was no significant difference among all the treatments for whole and sliced mushrooms.

Table 11. Effect of UV radiation and acetic acid preservative (1%) on change in ergosterol content (mg/g) of whole *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	0.52 ± 0.00^{Aa}	0.54 ± 0.00^{Aa}	0.53 ± 0.01^{Aa}	0.54 ± 0.01^{Aa}	0.53 ± 0.01^{Aa}	0.52 ± 0.01^{Aa}	0.54 ± 0.01^{Aa}
T_2	0.46 ± 0.01^{Ba}	0.46 ± 0.01^{Ba}	0.46 ± 0.00^{Ba}	0.47 ± 0.01^{Ba}	0.46 ± 0.01^{Ba}	0.47 ± 0.00^{Ba}	0.46 ± 0.00^{Ba}
T_3	0.47 ± 0.01^{Ba}	0.46 ± 0.01^{Ba}	0.47 ± 0.01^{Ba}	0.46 ± 0.01^{Ba}	0.47 ± 0.01^{Ba}	0.47 ± 0.00^{Ba}	0.47 ± 0.00^{Ba}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 12. Effect of UV radiation and acetic acid preservative (1%) on change in ergosterol content (mg/g) of sliced *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	0.56 ± 0.00^{Aa}	0.57 ± 0.00^{Aa}	0.56 ± 0.01^{Aa}	0.56 ± 0.00^{Aa}	0.57 ± 0.00^{Aa}	0.57 ± 0.01^{Aa}	0.57 ± 0.00^{Aa}
T_2	0.45 ± 0.01^{Ba}	0.45 ± 0.00^{Ba}	0.44 ± 0.00^{Ba}	0.45 ± 0.01^{Ba}	0.45 ± 0.00^{Ba}	0.45 ± 0.00^{Ba}	0.46 ± 0.01^{Ba}
T_3	0.46 ± 0.01^{Ba}	0.45 ± 0.00^{Ba}	0.45 ± 0.01^{Ba}	0.45 ± 0.01^{Ba}	0.46 ± 0.01^{Ba}	0.45 ± 0.01^{Ba}	0.44 ± 0.01^{Ba}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

Table 13. Effect of UV radiation and acetic acid preservative (1%) on change in protein content (%) of whole *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	3.46 ± 0.04^{Aa}	3.24 ± 0.04^{Bb}	3.19 ± 0.04^{Bb}	3.04 ± 0.04^{Cc}	2.90 ± 0.04^{Cd}	2.87 ± 0.03^{Cd}	2.85 ± 0.03^{Cd}
T_2	3.42 ± 0.04^{Aa}	3.40 ± 0.04^{Aa}	3.33 ± 0.02^{Aab}	3.28 ± 0.02^{Bbc}	3.26 ± 0.02^{Bbc}	3.23 ± 0.01^{Bc}	3.20 ± 0.02^{Bc}
T_3	3.45 ± 0.05^{Aa}	3.42 ± 0.04^{Aa}	3.41 ± 0.03^{Aa}	3.41 ± 0.03^{Aa}	3.38 ± 0.01^{Aa}	3.38 ± 0.01^{Aa}	3.35 ± 0.01^{Aa}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 14. Effect of UV radiation and acetic acid preservative (1%) on change in protein content (%) of sliced *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	3.89 ± 0.05^{Aa}	3.53 ± 0.04^{Bb}	3.39 ± 0.04^{Cb}	3.16 ± 0.05^{Cc}	3.02 ± 0.06^{Ccd}	2.94 ± 0.03^{Cd}	2.87 ± 0.05^{Cd}
T_2	3.84 ± 0.06^{Aa}	3.74 ± 0.07^{ABab}	3.60 ± 0.05^{Bbc}	3.50 ± 0.04^{Bcd}	3.36 ± 0.04^{Bde}	3.24 ± 0.04^{Bef}	3.13 ± 0.04^{Bf}
T_3	3.88 ± 0.07^{Aa}	3.86 ± 0.09^{Aa}	3.86 ± 0.06^{Aa}	3.84 ± 0.05^{Aa}	3.83 ± 0.05^{Aa}	3.83 ± 0.06^{Aa}	3.83 ± 0.05^{Aa}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

During storage, the protein content of whole mushrooms decreased in T_1 (whole mushroom stored at 4℃ (Control)) from 3.46 per cent to 2.85 per cent, while T_2 (UV induced whole mushroom stored at 4℃) showed a slight reduction from 3.42 per cent to 3.20 per cent (Table 13). For sliced mushrooms, protein content declined in T_1(sliced mushroom stored at 4℃ (Control)) from 3.89 per cent to 2.87 per cent, and T_2 (UV induced sliced mushroom stored at 4℃) showed a decrease from 3.84 per cent to 3.13 per cent (Table 14). This decline may be attributed to the activation of protease enzymes during storage, which leads to reduced protein levels and an increase in free amino acids (Murr and Morris, 1975; Rai and Saxena, 1989). The activity of hydrolytic enzymes such as protease and tyrosinase during storage breaks down proteins, causing a decrease in total protein content (Rai and Arumuganathan, 2003). The results obtained in the study, showed that there was no significant reduction of protein in UV induced mushrooms treated with 1% acetic acid and this was in consistent with Aadhilakshmi (2022) who investigated the effect of 1% acetic acid on extending the shelf life of *Pleurotus eous* during storage. Although there was similar change between untreated and treated samples, protein content of treated samples was significantly higher than untreated samples during entire storage period (Liao *et al*., 2016). Thus, mushrooms treated with UV-C and acetic acid shows lesser reduction of protein during storage.

4.2.1.4 Carbohydrates

Carbohydrates are a crucial component of mushrooms, contributing to their energy content and overall nutritional value. The carbohydrate content of the mushroom during the course of storage was shown in Table 15 and 16. On a fresh weight basis, the initial carbohydrate content ranged from 3.45 to 3.47 per cent (For whole mushroom) and 4.28 to 4.31 per cent (for sliced mushroom), there was no significant difference among all the treatments for all the three forms (Whole, sliced and powdered).

During storage, the carbohydrate content of whole mushrooms decreased in T_1 (whole mushroom stored at 4℃ (Control)) from 3.45 per cent to 2.02 per cent, while T_2 (UV induced whole mushroom stored at 4℃) exhibited a slight reduction from 3.46 per cent to 2.46 per cent (Table 15). For sliced mushrooms, the carbohydrate content in T_1

Table 15. Effect of UV radiation and acetic acid preservative (1%) on change in carbohydrate content (%) of whole *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	3.45 ± 0.04^{Aa}	3.39 ± 0.04^{Aa}	3.11 ± 0.04^{Bb}	2.80 ± 0.03^{Cc}	2.58 ± 0.03^{Cd}	2.26 ± 0.02^{Ce}	2.02 ± 0.02^{Cf}
T_2	3.46 ± 0.04^{Aa}	3.45 ± 0.04^{Aa}	3.24 ± 0.04^{Bb}	3.15 ± 0.04^{Bb}	2.95 ± 0.03^{Bc}	2.65 ± 0.03^{Bd}	2.46 ± 0.03^{Be}
T_3	3.47 ± 0.05^{Aa}	3.46 ± 0.05^{Aa}	3.44 ± 0.05^{Aa}	3.46 ± 0.05^{Aa}	3.45 ± 0.056^{Aa}	3.46 ± 0.05^{Aa}	3.44 ± 0.05^{Aa}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 16. Effect of UV radiation and acetic acid preservative (1%) on change in carbohydrate content (%) of sliced *Pleurotus eous* during storage period

Treatments	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	4.28 ± 0.05^{Aa}	4.25 ± 0.05^{Aa}	4.14 ± 0.05^{Aa}	3.92 ± 0.05^{Bb}	3.72 ± 0.05^{Bc}	3.51 ± 0.05^{Cd}	3.20 ± 0.05^{Ce}
T_2	4.30 ± 0.05^{Aa}	4.29 ± 0.05^{Aa}	4.18 ± 0.05^{Aab}	4.10 ± 0.05^{Bb}	3.85 ± 0.06^{Bc}	3.72 ± 0.06^{Bc}	3.51 ± 0.04^{Bd}
T_3	4.31 ± 0.06^{Aa}	4.31 ± 0.06^{Aa}	4.31 ± 0.06^{Aa}	4.30 ± 0.06^{Aa}	4.31 ± 0.05^{Aa}	4.30 ± 0.05^{Aa}	4.29 ± 0.08^{Aa}

Note: Values are given as mean ± standard error. Means with same superscript, in a column (lower case) and row (upper case) do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

(sliced mushrooms stored at 4°C, Control) dropped from 4.28 per cent to 3.20 per cent and T_2 (UV induced sliced mushroom stored at 4°C) showed a decrease from 4.30 per cent to 3.51 per cent (Table 16). This decline may be attributed to the activation of amylase and other hydrolytic enzymes which can degrade complex carbohydrates into simpler sugars (Murr and Morris, 1975).

Microbial growth on stored mushrooms can also contribute to carbohydrate loss. Microorganisms such as bacteria and fungi can ferment carbohydrates, converting them into gases and other by-products (Rai and Saxena, 1989). The results indicated that there was no significant reduction in carbohydrate content in UV-induced mushrooms treated with 1% acetic acid. Although the changes in carbohydrate levels were similar between untreated and treated samples, the carbohydrate content in the treated samples remained significantly higher than in the untreated samples throughout the entire storage period (Liao *et al*., 2016). Therefore, mushrooms treated with UV-C and acetic acid experienced a lesser reduction in carbohydrate content during storage.

4.2.1.5 Minerals

There was no significant change in the all the minerals (*viz*., phosphorous, potassium, iron, manganese, copper and zinc) of whole and sliced mushroom during storage (Table 17 to 22). This could be due to many minerals do not react readily with moisture, light, or air. This low reactivity further contributes to their stability during storage, allowing them to retain their mineral content without significant changes (Kortei *et al*., 2017).

4.2.2 Effect of UV radiation and acetic acid preservative (1%) on physical properties of whole and sliced *Pleurotus eous* mushroom during storage

4.2.2.1 Physiological weight loss

The weight loss of whole and sliced mushrooms is illustrated in Figures 7 and 8. For whole mushrooms, weight loss significantly increased from 0 per cent to 4.95 per cent in T_1 (whole mushrooms stored at 4°C, Control). In T_2 (UV-induced whole mushrooms

Table 17. Effect of UV radiation and acetic acid preservative (1%) on change in phosphorous content (%) of whole and sliced *Pleurotus eous* during storage period

Treatments	Whole							Sliced						
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	1.44	1.41	1.45	1.41	1.43	1.52	1.41	1.99	2.22	2.10	2.10	1.94	1.97	1.99
T_2	1.44	1.45	1.45	1.45	1.42	1.50	1.43	2.01	2.23	2.20	1.97	2.05	2.02	2.01
T_3	1.43	1.46	1.41	1.44	1.44	1.53	1.43	2.02	2.20	2.09	2.08	2.08	2.04	2.03
SE(m)	0.029	0.023	0.022	0.03	0.021	0.023	0.022	0.041	0.033	0.036	0.041	0.031	0.033	0.041
C.D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 18. Effect of UV radiation and acetic acid preservative (1%) on change in potassium content (%) of whole and sliced *Pleurotus eous* during storage period

Treatments	Whole							Sliced						
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	3.63	3.57	3.55	3.54	3.63	3.55	3.57	3.66	3.51	3.53	3.58	3.55	3.56	3.67
T_2	3.65	3.55	3.55	3.55	3.67	3.57	3.61	3.67	3.52	3.54	3.58	3.56	3.61	3.66
T_3	3.62	3.54	3.57	3.56	3.65	3.58	3.67	3.64	3.53	3.51	3.57	3.60	3.63	3.65
SE(m)	0.077	0.054	0.059	0.075	0.055	0.06	0.078	0.074	0.053	0.059	0.072	0.057	0.058	0.074
C.D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

Table 19. Effect of UV radiation and acetic acid preservative (1%) on change in iron content (ppm) of whole and sliced *Pleurotus eous* during storage period

Treatments	Whole							Sliced						
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	451.80	446.00	447.40	460.40	445.60	452.80	452.60	434.40	446.80	439.20	443.60	445.00	447.60	437.80
T_2	452.20	448.20	452.20	442.40	449.20	450.80	451.60	433.20	441.20	433.80	443.20	430.60	443.80	443.80
T_3	452.00	465.60	451.80	459.60	446.80	451.80	453.20	434.80	444.80	440.40	437.00	430.40	437.80	461.00
SE(m)	9.553	6.853	7.529	9.599	6.734	7.562	9.565	6.709	3.576	5.289	7.707	6.537	6.843	9.147
C.D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 20. Effect of UV radiation and acetic acid preservative (1%) on change in manganese content (ppm) of whole and sliced *Pleurotus eous* during storage period

Treatments	Whole							Sliced						
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	34.80	32.60	32.00	32.80	32.60	32.20	34.20	30.60	29.20	30.60	29.20	29.40	29.60	27.80
T_2	34.20	34.80	33.20	33.20	33.40	33.60	35.00	29.60	29.40	30.80	27.40	30.20	29.20	29.60
T_3	34.60	33.80	32.20	33.80	33.00	32.40	34.00	30.00	28.80	30.20	28.10	29.60	30.40	27.80
SE(m)	0.729	0.509	0.543	0.706	0.497	0.548	0.518	0.606	0.376	0.388	0.567	0.448	0.496	0.576
C.D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

Table 21. Effect of UV radiation and acetic acid preservative (1%) on change in copper content (ppm) of whole and sliced *Pleurotus eous* during storage period

Treatments	Whole							Sliced						
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	32.20	32.80	33.00	33.40	31.60	33.10	31.40	36.20	36.60	36.00	32.60	34.00	35.80	35.80
T_2	32.00	31.80	32.60	33.80	31.80	32.60	32.00	37.40	37.00	36.40	32.20	34.40	35.00	35.20
T_3	32.80	33.60	31.80	32.40	32.40	31.80	31.60	36.80	36.20	35.80	33.20	33.80	35.20	35.20
SE(m)	0.488	0.546	0.487	0.558	0.482	0.547	0.477	0.748	0.55	0.533	0.577	0.537	0.546	0.791
C.D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 22. Effect of UV radiation and acetic acid preservative (1%) on change in zinc content (ppm) of whole and sliced *Pleurotus eous* during storage period

Treatments	Whole							Sliced						
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T_1	231.80	239.00	230.80	231.60	232.80	236.60	232.80	292.20	299.60	296.40	293.40	291.40	293.00	286.80
T_2	236.80	237.40	237.40	231.20	234.60	235.80	234.80	302.40	292.40	297.40	290.40	292.40	290.80	304.80
T_3	238.80	239.20	234.80	230.80	231.60	236.20	232.20	299.20	305.60	295.80	292.40	291.80	292.20	303.60
SE(m)	3.559	3.991	3.533	3.87	3.506	3.953	3.905	6.062	4.515	4.963	5.909	4.395	4.887	4.962
C.D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Treatment details:
T_1: Raw mushroom stored at 4°C (Control) T_2: UV induced mushroom stored at 4°C
T_3: UV induced mushroom pre-treated with 1 % Acetic acid and stored at 4°C

stored at 4°C), weight loss ranged from 3.97 per cent to 6.64 per cent, while T_3 (UV-induced whole mushrooms treated with 1% acetic acid and stored at 4°C) exhibited weight loss ranging from 3.94 per cent to 5.38 per cent over the entire storage period. A similar pattern was observed for sliced mushrooms. Weight loss in T_1 (sliced mushrooms stored at 4°C, Control) increased significantly from 0 per cent to 5.10 per cent. In T_2 (UV-induced sliced mushrooms stored at 4°C), weight loss ranged from 5.24 per cent to 8.91 per cent, and T_3 (UV-induced sliced mushrooms treated with 1% acetic acid and stored at 4°C) showed a weight loss ranging from 5.24 per cent to 6.83 per cent throughout the storage period.

Physiological weight loss is due to fast dehydration by loss of water. This is because mushrooms are coated with a thin epidermal structure, in addition to having high rates of respiration and transpiration (Nasiri *et al*., 2017). In both whole and sliced mushroom, the maximum weight loss was observed in T_1 and minimum weight loss was observed in T_3. This difference may be attributed to UV-C irradiation, which effectively reduces weight loss by minimizing dehydration during storage. UV-C radiation can inhibit enzymes responsible for browning and degradation, such as polyphenol oxidase (PPO). By reducing enzymatic activity, UV-C treatment helps preserve the mushrooms' texture and moisture content, leading to less weight loss (Nunes *et al*., 2024). Aadhilakshmi (2022) studied the impact of 1% acetic acid on reducing physiological weight loss in *Pleurotus eous* mushrooms during storage. The research revealed that mushrooms treated with acetic acid exhibited a significantly lower weight loss compared to the control. This is likely due to acetic acid’s ability to create an acidic environment that hinders microbial growth and enzymatic activity, both of which contribute to the weight loss of mushrooms.

4.2.3 Effect of UV radiation and acetic acid preservative (1%) and on microbial population in whole and sliced *Pleurotus eous* mushroom during storage

4.2.3.1 Bacteria

Total aerobic plate counts were performed using pour plates of plate count agar to assess the effects of UV radiation and acetic acid on the microbial load of *Pleurotus eous*

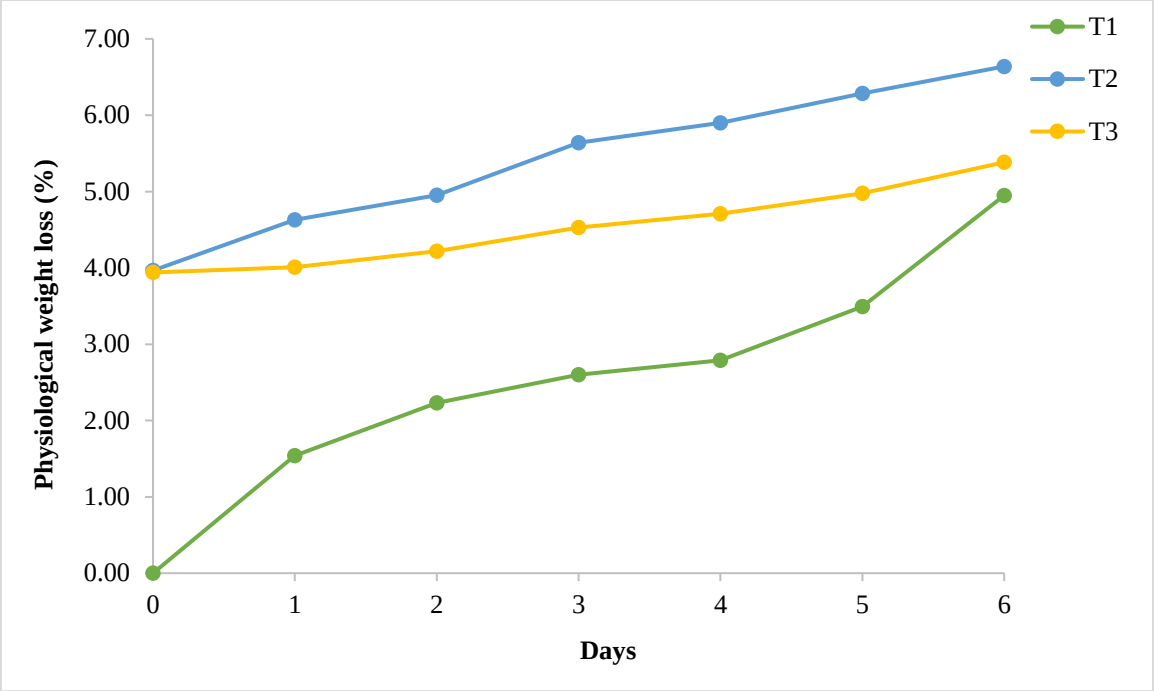


Fig. 7. Effect of UV radiation and acetic acid preservative (1%) on change in physiological weight loss (%) of whole *Pleurotus eous* during storage period

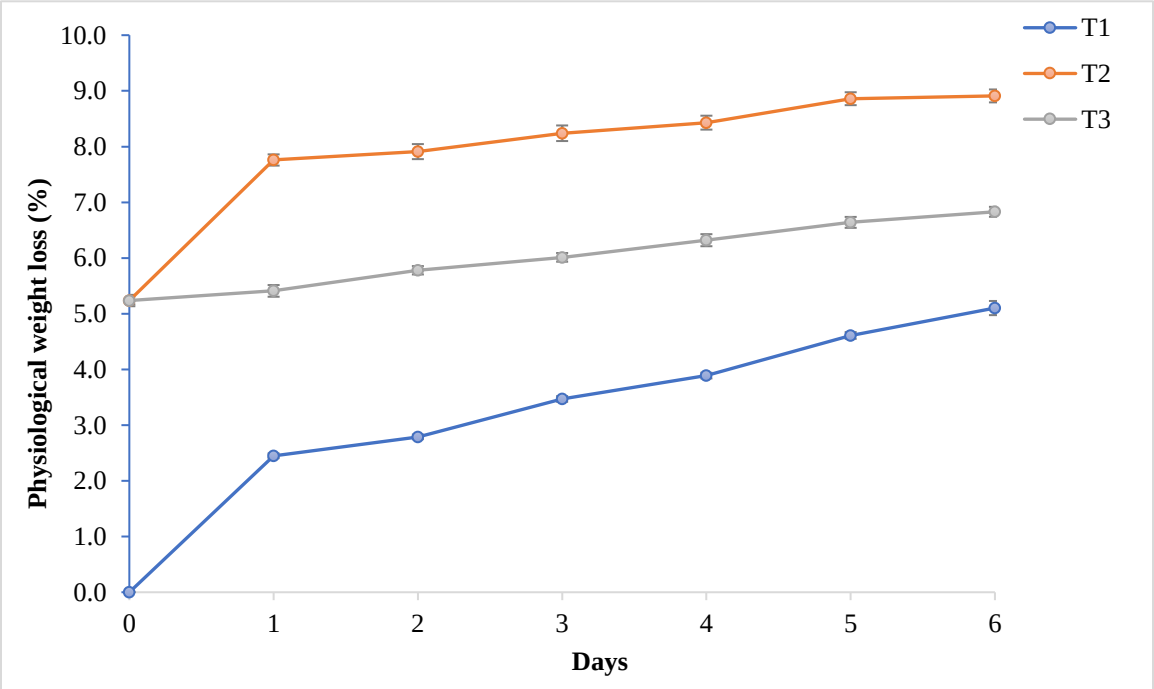


Fig. 8. Effect of UV radiation and acetic acid preservative (1%) on change in physiological weight loss (%) of sliced *Pleurotus eous* during storage period

during storage. Bacterial counts were recorded daily, showing an increase in bacterial populations with prolonged storage. The results indicated a notable rise in bacterial counts (cfu/g) across all mushroom forms.

Fig. 9a. illustrates the bacterial population of whole mushrooms during storage. On the 6th day of storage, treatment T_3 (UV-induced whole mushrooms treated with 1% acetic acid and stored at 4°C) had the lowest bacterial growth (4.20×10^4 cfu/g), while treatment T_1 (whole mushrooms stored at 4°C, Control) exhibited the highest bacterial growth (12.60×10^4 cfu/g). The population in T_2 (UV-induced whole mushrooms stored at 4°C) was 6.80×10^4 cfu/g. A similar trend was observed with sliced mushroom, as shown in Fig. 9b. The highest bacterial growth was recorded in T_1 (sliced mushrooms stored at 4°C, Control :14.40×10^4 cfu/g), followed by T_2 (UV-induced sliced mushrooms stored at 4°C: 7.60×10^4 cfu/g) and lowest count was in T_3 (UV-induced sliced mushrooms treated with 1% acetic acid and stored at 4°C: 4.40×10^4 cfu/g).

According to the FSSAI, 2023 the aerobic microbial count was within the limit in T_3 in whole and sliced mushroom for all the treatments. This may be due to organic acids has an important role in antimicrobial property, therefore there was a reduction in growth in the samples treated with acetic acid (Kamal *et al*., 2022). The bacterial population in UV-treated samples was significantly lower compared to the control. This reduction can be attributed to the lower initial bacterial load due to the inactivation of bacteria by UV radiation. UV exposure damages the nucleic acids (DNA and RNA) of bacterial cells, as the nucleotides absorb UV light. This damage disrupts bacterial replication and survival, effectively reducing the population during storage (Guan *et al*., 2012).

4.2.3.2 Yeast and mold

The number of fungal colonies inside the mushrooms increased over time, leading to a decline in quality (Shao *et al*., 2021). When the development of fungi progresses rapidly, yeast and mold may become visible on the surface of food items without further inspection. Nonetheless, the use of various preservatives can significantly affect the preservation of yeast and mold. Fig.10a. illustrates the yeast and mold population of whole mushrooms during storage. On the 6th day of storage, treatment T_3 (UV-induced whole

mushrooms treated with 1% acetic acid and stored at 4°C) had the lowest yeast and mold growth (4.80×10^2 cfu/g), while treatment T_1 (whole mushrooms stored at 4°C, Control) exhibited the highest yeast and mold growth (9.40×10^2 cfu/g). The population in T_2 (UV-induced whole mushrooms stored at 4°C) was 5.80×10^2 cfu/g. A similar trend was observed with sliced mushroom, as shown in Fig. 10b. The highest yeast and mold growth was recorded in T_1 (sliced mushrooms stored at 4°C, Control :11.60×10^2 cfu/g), followed by T_2 (UV-induced sliced mushrooms stored at 4°C: 6.60×10^2 cfu/g) and lowest count was in T_3 (UV-induced whole mushrooms treated with 1% acetic acid and stored at 4°C: 5.00×10^2 cfu/g).

According to the FSSAI, 2023 the yeast and mold count was within the limit in T_3 in whole and sliced mushroom for all the treatments. This may be due to organic acids has an important role in antimicrobial property, therefore there was a reduction in growth in the samples treated with acetic acid (Kamal *et al*., 2022). The yeast and mold population were less in case of UV treated samples compared to control, this may due to less population at the initial day due to inactivation of yeast and mold upon exposure (Guan *et al*., 2012)

4.2.4 Effect of UV radiation and acetic acid preservative (1%) on overall acceptability based on sensory evaluation of *Pleurotus eous* during storage period

The sensory evaluation of *Pleurotus eous* treated with UV radiation and acetic acid preservative (1%) revealed significant changes in overall acceptability. Organoleptic scores declined as storage time progressed. For whole mushrooms, T_3 (UV-induced whole mushrooms treated with 1% acetic acid and stored at 4°C: 6.28) received the highest sensory score up to six days of storage, followed by T_2 (UV-induced whole mushrooms stored at 4°C: 5.75), while T_1 (whole mushrooms stored at 4°C, Control: 5.41) had the lowest sensory score (Fig. 11). A similar pattern was observed in sliced mushrooms, where T_3 (UV-induced sliced mushrooms treated with 1% acetic acid and stored at 4°C: 6.48) scored highest and T_1 (sliced mushrooms stored at 4°C, Control: 5.32) scored the lowest (Fig. 12).

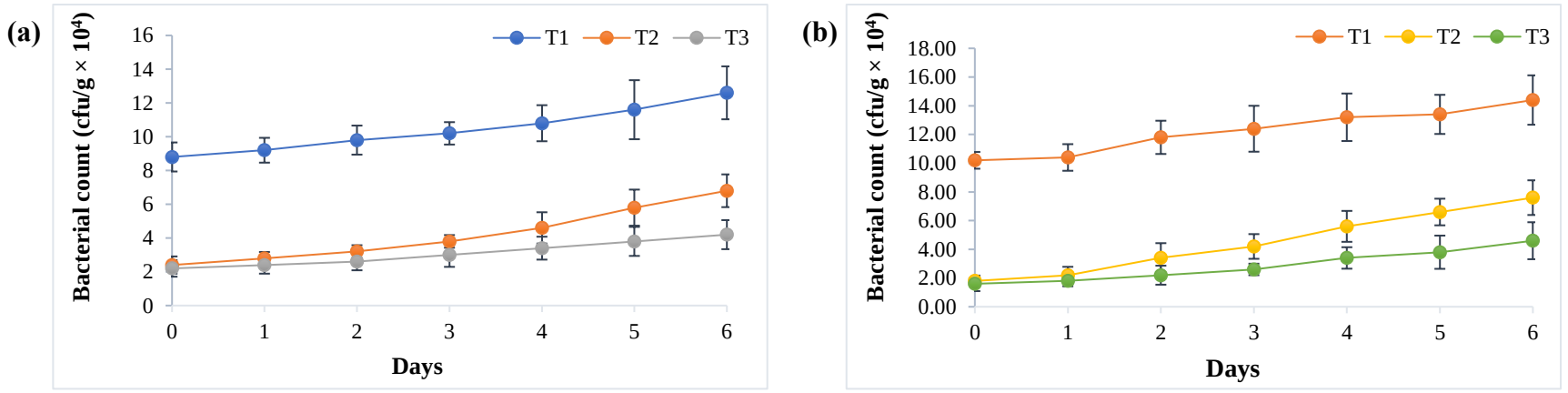


Fig. 9. Effect of UV radiation and acetic acid preservative (1%) on change in bacterial count of (a) whole and (b) sliced *Pleurotus eous* during storage period

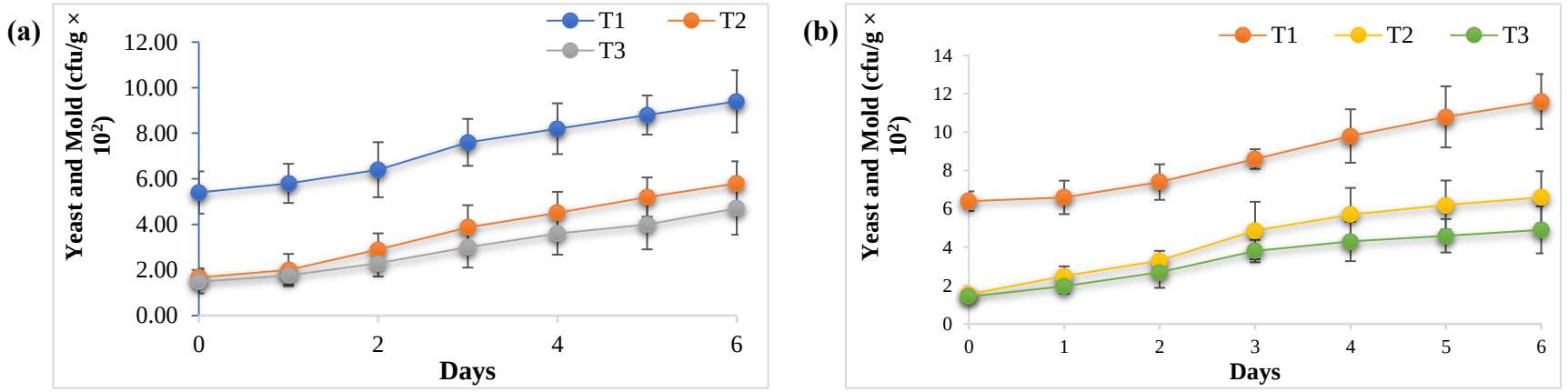


Fig. 10. Effect of UV radiation and acetic acid preservative (1%) on change in yeast or mold count of (a) whole and (b) sliced *Pleurotus eous* during storage period

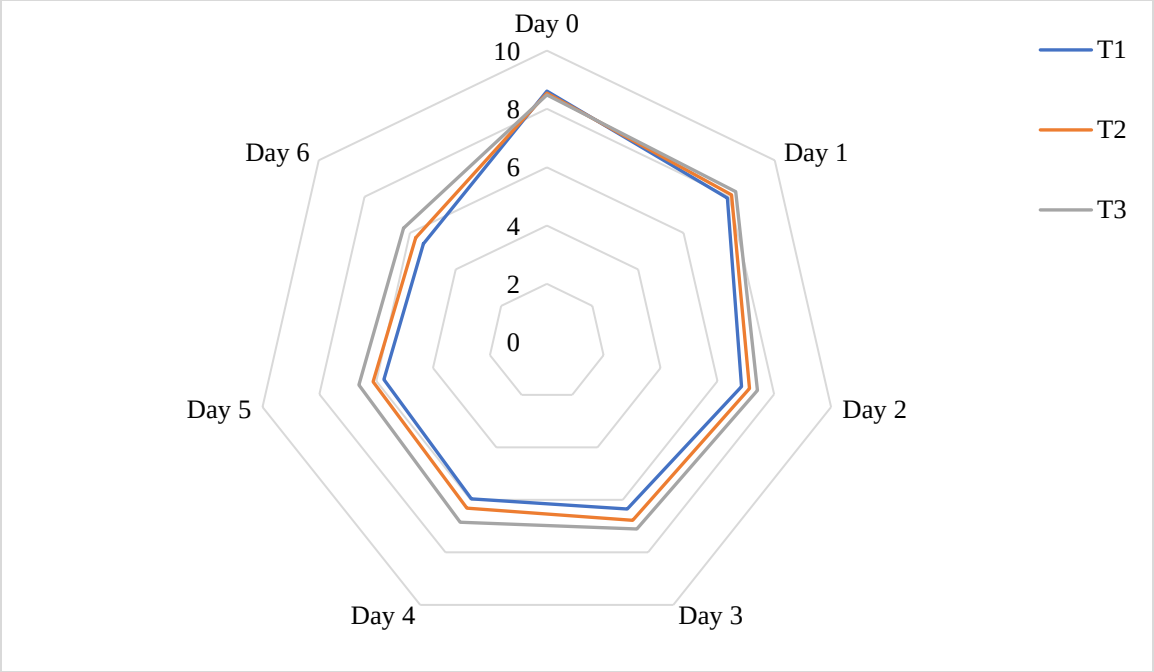


Fig. 11. Effect of UV radiation and acetic acid preservative (1%) on change in overall acceptability (based on Sensory evaluation) of whole *Pleurotus eous* during storage period

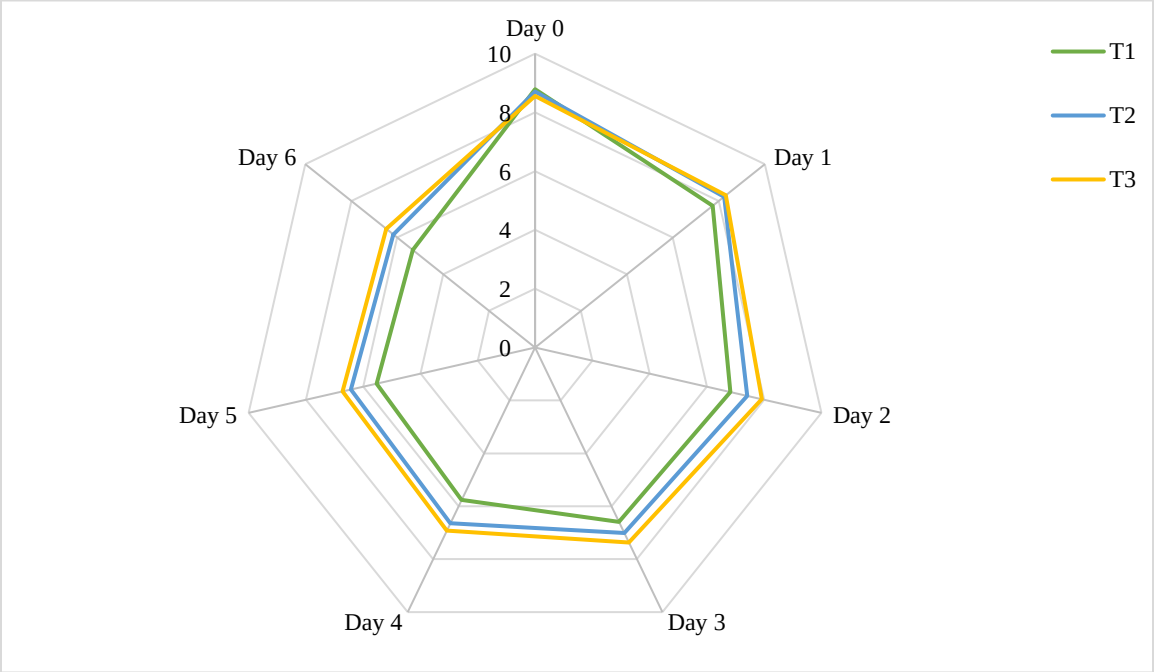


Fig. 12. Effect of UV radiation and acetic acid preservative (1%) on change in overall acceptability (based on Sensory evaluation) of sliced *Pleurotus eous* during storage period

Aadhilakshmi (2022) noted that acetic acid at a 1% concentration maintained consumer preference for up to six days, achieving an overall acceptability score of 6.61 based on a sensory evaluation using the 9-point hedonic scale. However, due to the bleaching properties of acids, the acetic acid-treated samples experienced greater color loss compared to other treatments, resulting in lower scores. Throughout the storage period, a marked decline in organoleptic quality was observed, which may be due to the development of unpleasant flavours, odours, and discoloration in mushrooms which are primarily influenced by biochemical changes that are highly temperature-dependent (Premakumar *et al*., 2020).

4.2.5 Effect of UV radiation and storage temperature on biochemical properties of powdered *Pleurotus eous* mushroom during storage

4.2.5.1 Vitamin D_2

In case of powdered mushroom, the Initial vitamin D_2 content was recorded as 9.34 µg/g in T_1 (Powdered mushroom stored at room temperature) and T_2 (Powdered mushroom stored at 4°C) and 250.56 µg/g in T_3 (UV induced powdered mushroom stored at room temperature) and T_4 (UV induced powdered mushroom stored at 4°C). During storage, vitamin D_2 concentrations remained relatively stable upto 30 days of storage at either 4 °C or room temperature when mushrooms were treated with UV-C irradiation (Fig. 13 a & b). Instead, there a slight increase in vitamin D_2 of mushroom powder stored at room temperature T_1 (9.91 µg/g) and T_3 (265.40 µg/g). This may be due to additional ergosterol conversion to vitamin D_2 in mushrooms exposed to the artificial light during storage (Koyyalamudi *et al*., 2009). According to Dawadi *et al*. (2022) the powdered mushroom can be stored up to 3 months without losing its quality and nutritional properties. The retention of the vitamin D_2 content in powdered mushroom stored up to 3 months was calculated from the equation presented in Fig. 14 (a) and (b). The vitamin D_2 content of UV induced powdered mushroom stored up to 3 months was 298.38 µg/g (For powdered mushroom stored at room temperature) and 236.15 µg/g (For powdered mushroom stored at 4°C).

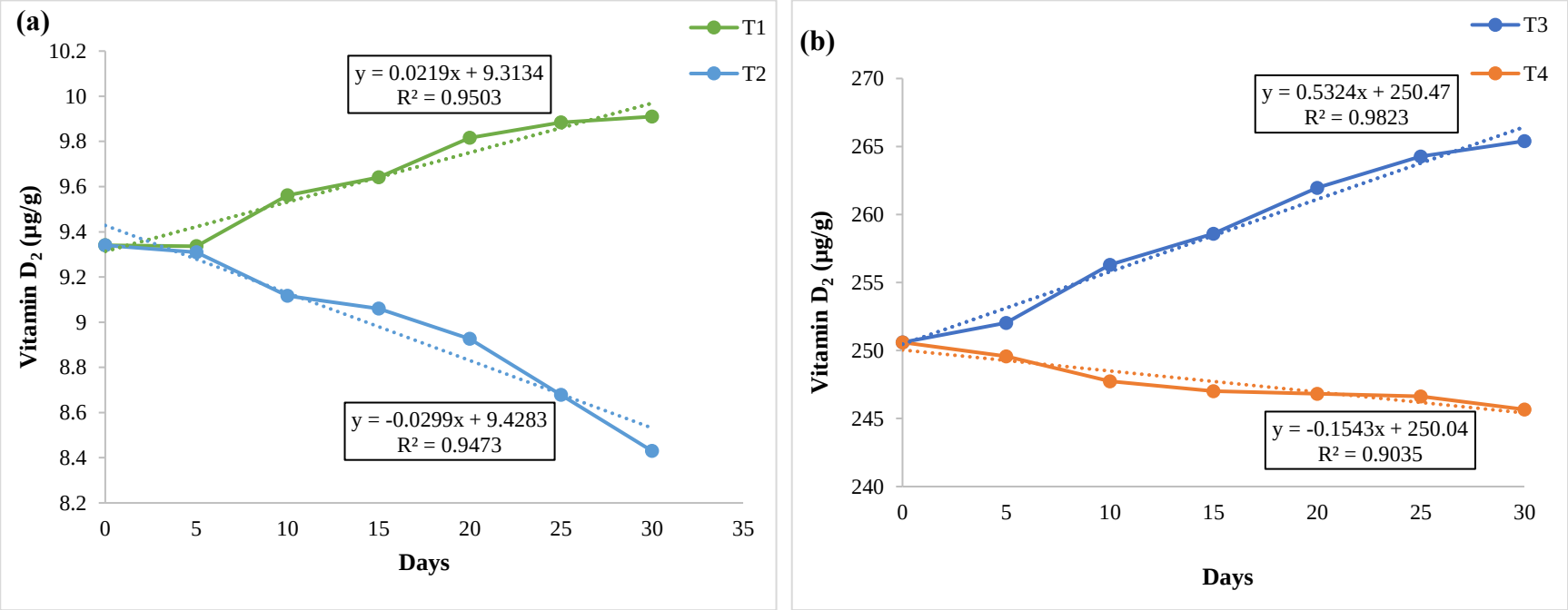


Fig. 13. Effect of UV radiation and storage temperature on change in (a) vitamin D_2 content (µg/g) of T_1 and T_2 (b) vitamin D_2 content (µg/g) of T_3 and T_4 of powdered *Pleurotus eous* during storage period

Treatment details:

T_1: Powdered mushroom stored at room temperature

T_2: Powdered mushroom stored at 4°C

T_3: UV induced powdered mushroom stored at room temperature

T_4: UV induced powdered mushroom stored at 4°C

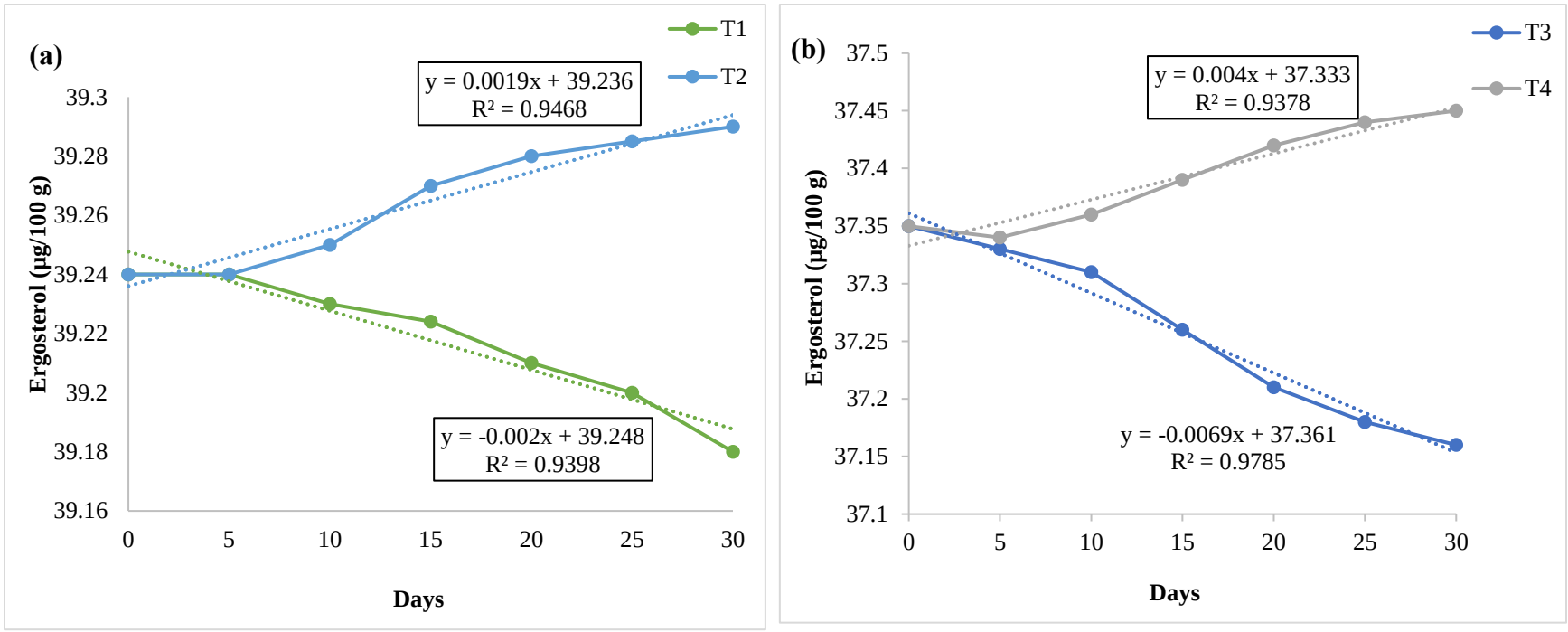


Fig. 14. Effect of UV radiation and storage temperature on change in ergosterol content (µg/100 g) of powdered *Pleurotus eous* during storage period

Treatment details:

T_1: Powdered mushroom stored at room temperature

T_2: Powdered mushroom stored at 4°C

T_3: UV induced powdered mushroom stored at room temperature

T_4: UV induced powdered mushroom stored at 4°C

4.2.5.2 Ergosterol

For powdered mushrooms, the Initial ergosterol content was recorded as 3924 µg/g in T_1 (Powdered mushroom stored at room temperature) and T_2 (Powdered mushroom stored at 4°C) and 3735 µg/g in T_3 (UV induced powdered mushroom stored at room temperature) and T_4 (UV induced powdered mushroom stored at 4°C). During storage upto 30 days, there was a slight decrease in the ergosterol content of T_1 (3918 µg/g) and T_3 (3716 µg/100g) (Fig. 14). This may be due to exposure to ambient light and oxygen at room temperature can cause the degradation of ergosterol through oxidation or conversion into vitamin D_2. Additionally, higher temperatures can accelerate chemical reactions, leading to faster breakdown compared to cooler storage conditions. This results in reduced ergosterol levels over time in room temperature storage (Guan *et al*., 2016).

4.2.5.3 Protein

Fig. 15. shows that over a period of storage upto 30 days. On dry weight basis, the initial protein content ranged from 25.54 to 26.38 per cent. There was no significant change in protein content of T_2 (Powdered *Pleurotus eous* stored at 4°C) and T_4 (UV exposed powdered *Pleurotus eous* stored at 4°C). This is attributed to reduced microbial growth and enzymatic activity, which collectively help sustain the quality of the powdered mushrooms over time (Gholami *et al*., 2017). In contrast, storing both treated and untreated powdered mushrooms at room temperature possess a decline in protein content from 26.38 to 23.89 per cent in T_1 (Powdered mushroom stored at room temperature) and from 25.54 to 24.86 per cent in T_3 (UV induced powdered mushroom stored at room temperature). This could be due to increased microbial activity and potential enzymatic browning, contributing to a significant loss of protein during this period (Yusof *et al*., 2022).

4.2.5.4 Carbohydrates

Fig. 16. shows that over a period of storage upto 30 days, there was no significant change in carbohydrate content of T_2 (Powdered *Pleurotus eous* stored at 4°C) and T_4 (UV exposed powdered *Pleurotus eous* stored at 4°C). This is attributed to reduced microbial growth and enzymatic activity, which collectively help sustain the quality of the powdered mushrooms over time (Gholami *et al*., 2017). In contrast, storing both treated and untreated

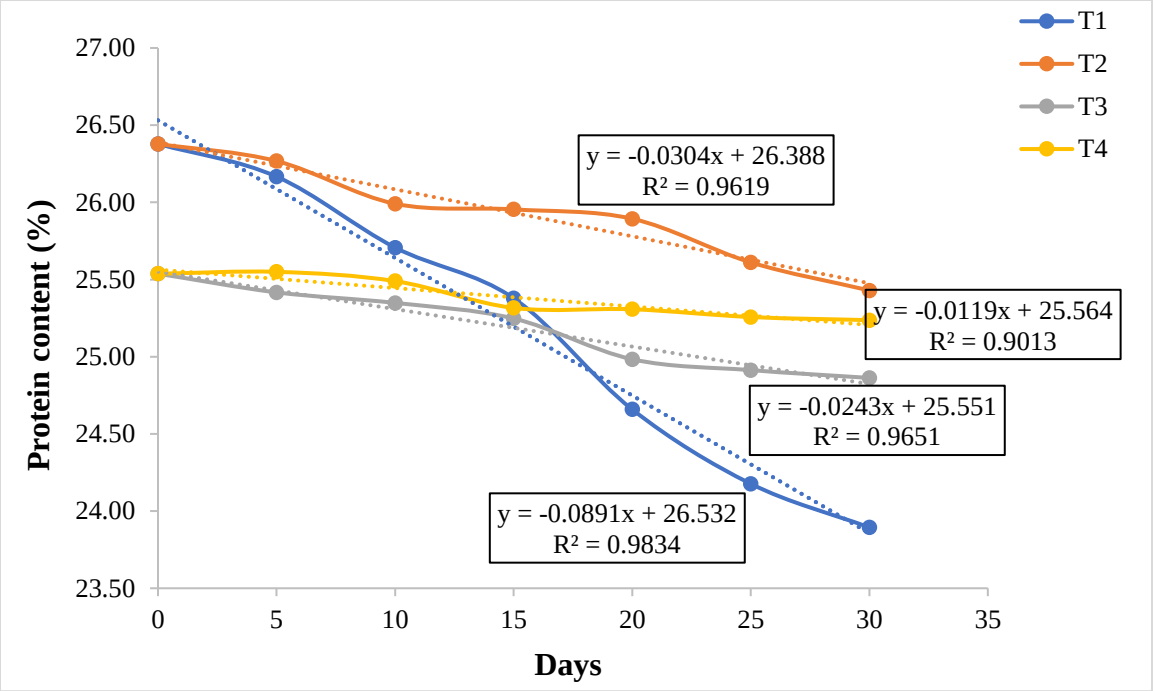


Fig. 15. Effect of UV radiation and storage temperature on change in protein content (%) of powdered *Pleurotus eous* during storage period

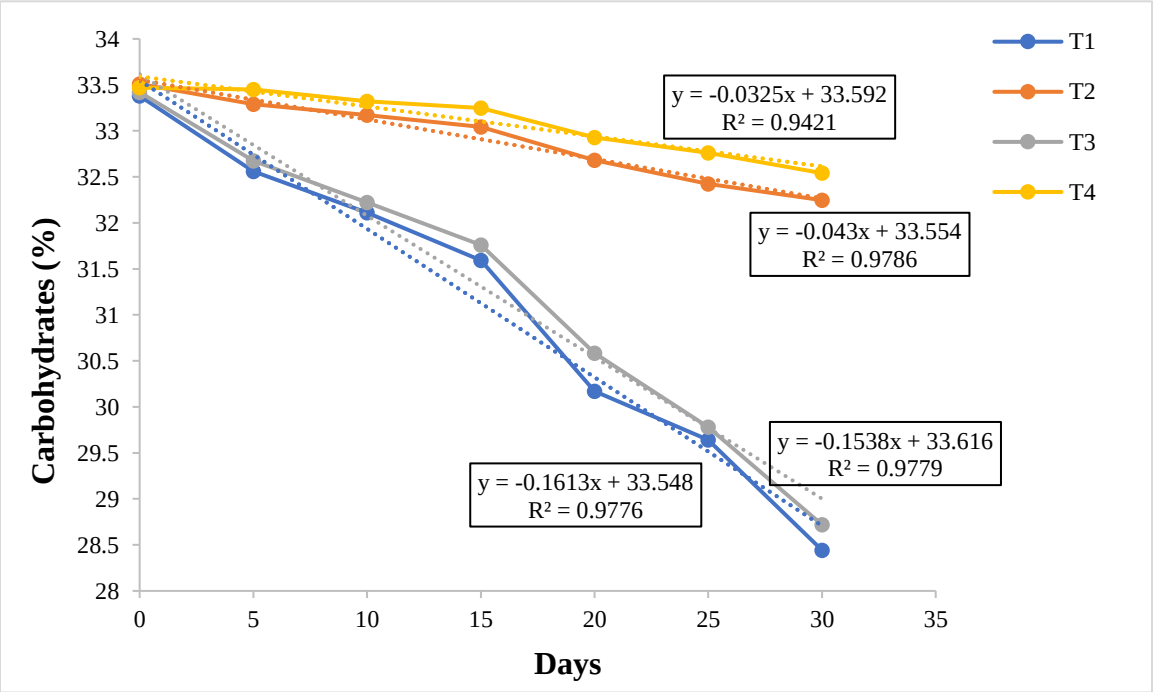


Fig. 16. Effect of UV radiation and storage temperature on change in carbohydrate content (%) of powdered *Pleurotus eous* during storage period

powdered mushrooms at room temperature possess a decline in carbohydrate content from 33.38 to 28.44 per cent in T_1 (Powdered mushroom stored at room temperature) and from 33.42 to 28.72 per cent in T_3 (UV induced powdered mushroom stored at room temperature). This could be due to increased microbial activity and potential enzymatic browning, contributing to a significant loss of carbohydrate during this period (Yusof *et al*., 2022).

4.2.5.5 Minerals

There was no significant change in the all the minerals (viz., phosphorous, potassium, iron, manganese, copper and zinc) of powdered mushroom during storage (Table 23 to 28). This may be attributed to the fact that many minerals do not readily react with moisture, light, or air, which helps maintain their stability during storage. Their low reactivity ensures that the mineral content remains consistent over time, without undergoing significant changes (Kortei *et al*., 2017).

4.2.6 Effect of UV radiation and storage temperature on microbial population in powdered *Pleurotus eous* mushroom during storage

4.2.6.1 Bacteria

In case of powdered mushroom, the highest bacterial population was observed in T_1 (Powdered mushroom stored at room temperature) with 13.75×10^4 cfu/g, followed by T_2 (Powdered mushroom stored at 4°C) with 10.75×10^4 cfu/g and T_3 (UV induced powdered mushroom stored at room temperature) with 8.50×10^4 cfu/g and lowest population was observed in T_4 (UV induced powdered mushroom stored at 4°C) at 5.75×10^4 cfu/g (Fig. 17).

4.2.6.2 Yeast and mold

For powdered mushroom, the highest yeast and mold population was observed in T_1 (Powdered mushroom stored at room temperature) with 6.25×10^2 cfu/g, followed by T_2 (Powdered mushroom stored at 4°C) with 5.00×10^2 cfu/g and T_3 (UV induced powdered mushroom stored at room temperature) with 4.50×10^2 cfu/g and lowest population was

Table 23. Effect of UV radiation and storage temperature on change in phosphorous content (%) of powdered *Pleurotus eous* during storage period

Treatments	Initial	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
T_1	2.20±0.01	2.18±0.03	2.19±0.03	2.19±0.02	2.19±0.01	2.18±0.03	2.18±0.01
T_2	2.18±0.04	2.17±0.02	2.21±0.03	2.21±0.02	2.18±0.02	2.21±0.03	2.19±0.02
T_3	2.20±0.02	2.18±0.04	2.18±0.02	2.22±0.03	2.22±0.03	2.16±0.03	2.19±0.03
T_4	2.19±0.02	2.19±0.01	2.19±0.03	2.20±0.03	2.22±0.03	2.18±0.03	2.20±0.03
SE(m)	0.027	0.032	0.033	0.032	0.028	0.035	0.028
C.D.	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 24. Effect of UV radiation and storage temperature on change in potassium content (%) of powdered *Pleurotus eous* during storage period

Treatments	Initial	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
T_1	3.65±0.06	3.61±0.06	3.58±0.05	3.58±0.06	3.52±0.06	3.51±0.05	3.52±0.06
T_2	3.59±0.06	3.60±0.05	3.59±0.05	3.54±0.06	3.52±0.05	3.50±0.05	3.47±0.06
T_3	3.62±0.05	3.60±0.06	3.59±0.05	3.56±0.05	3.51±0.06	3.50±0.05	3.52±0.05
T_4	3.64±0.05	3.59±0.05	3.58±0.06	3.62±0.05	3.51±0.05	3.50±0.06	3.53±0.05
SE(m)	0.061	0.062	0.058	0.061	0.06	0.057	0.06
C.D.	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Treatment details:

T_1: Powdered mushroom stored at room temperature

T_2: Powdered mushroom stored at 4°C

T_3: UV induced powdered mushroom stored at room temperature

T_4: UV induced powdered mushroom stored at 4°C

Table 25. Effect of UV radiation and storage temperature on change in iron content (ppm) of powdered *Pleurotus eous* during storage period

Treatments	Initial	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
T_1	444.35±8.01	446.70±8.05	440.62±7.20	453.86±8.18	445.13±3.87	447.21±7.31	450.03±8.12
T_2	438.31±8.16	447.08±7.31	452.58±6.57	444.23±3.61	434.59±7.10	446.60±1.57	441.46±1.07
T_3	442.65±7.23	453.94±5.01	455.93±7.45	442.61±7.23	446.76±2.11	435.53±6.95	444.26±7.26
T_4	446.17±6.48	456.96±7.47	446.56±2.11	446.46±2.48	446.08±7.29	447.45±3.54	441.12±6.41
SE(m)	7.507	7.062	6.23	5.888	5.55	5.408	6.343
C.D.	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 26. Effect of UV radiation and storage temperature on change in manganese content (ppm) of powdered *Pleurotus eous* during storage period

Treatments	Initial	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
T_1	30.50±0.55	31.71±0.57	31.79±0.27	31.83±0.34	31.99±0.51	31.85±0.60	31.92±0.19
T_2	31.83±0.59	32.44±0.53	31.91±0.46	30.64±0.57	32.10±0.31	31.88±0.15	32.03±0.59
T_3	31.83±0.52	32.32±0.56	31.95±0.29	31.74±0.42	31.97±0.27	32.10±0.21	31.99±0.13
T_4	32.19±0.46	31.38±0.49	31.96±0.42	31.86±0.14	32.15±0.20	31.98±0.21	31.93±0.39
SE(m)	0.535	0.542	0.375	0.403	0.346	0.346	0.377
C.D.	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Treatment details:

T_1: Powdered mushroom stored at room temperature

T_2: Powdered mushroom stored at 4°C

T_3: UV induced powdered mushroom stored at room temperature

T_4: UV induced powdered mushroom stored at 4°C

Table 27. Effect of UV radiation and storage temperature on change in copper content (ppm) of powdered *Pleurotus eous* during storage period

Treatments	Initial	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
T_1	40.60±0.73	40.75±0.58	39.69±0.64	39.67±0.30	40.36±0.72	39.73±0.15	39.88±0.36
T_2	39.30±0.46	41.28±0.67	41.19±0.59	41.08±0.60	41.59±0.51	40.19±0.58	39.79±0.74
T_3	39.89±0.65	40.95±0.61	40.07±0.65	39.87±0.65	40.18±0.70	39.34±0.47	39.64±0.79
T_4	40.07±0.57	41.07±0.67	41.59±0.73	40.79±0.59	41.16±0.59	40.46±0.03	41.90±0.46
SE(m)	0.614	0.638	0.66	0.55	0.643	0.385	0.619
C.D.	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 28. Effect of UV radiation and storage temperature on change in zinc content (ppm) of powdered *Pleurotus eous* during storage period

Treatments	Initial	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
T_1	245.81±4.43	245.81±4.43	239.56±3.91	246.82±4.45	234.70±4.23	245.60±4.01	239.95±4.32
T_2	239.71±4.46	247.02±4.04	242.78±3.52	246.51±4.59	247.42±4.04	240.79±3.49	245.12±4.56
T_3	248.43±4.06	248.23±4.35	240.97±3.94	247.22±4.04	243.99±4.28	242.99±3.97	247.62±4.05
T_4	242.78±3.52	241.77±3.95	249.75±4.38	246.21±3.57	245.14±4.00	240.69±4.22	243.62±3.54
SE(m)	4.141	4.201	3.954	4.186	4.145	3.937	4.139
C.D.	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Treatment details:

T_1: Powdered mushroom stored at room temperature

T_2: Powdered mushroom stored at 4°C

T_3: UV induced powdered mushroom stored at room temperature

T_4: UV induced powdered mushroom stored at 4°C

observed in T_4 (UV induced powdered mushroom stored at 4℃) at 3.75×10^2 cfu/g (Fig. 18).

4.2.7 Effect of UV radiation and storage temperature on overall acceptability based on sensory evaluation of powdered *Pleurotus eous* during storage period

The sensory evaluation of *Pleurotus eous* s treated with UV radiation and storage temperature revealed no significant changes in overall acceptability (Fig. 19). The initial sensory scores of all the treatments are on par with each other. The highest mean value was recorded in treatment T_4 (UV induced powdered mushroom stored at 4℃) with 8.58, followed by T_2 (Powdered mushroom stored at 4℃) with 8.56 and T_3 (UV induced powdered mushroom stored at room temperature) with 8.31 and lowest mean value was observed in T_1 (Powdered mushroom stored at room temperature) with 8.18. On 30^{th} day, there was no significant change in sensory score of T_2 (8.50) and T_4 (8.54), whereas there is a slight decrease in the sensory score of T_1 (8.18) and T_3 (8.23).

The current study confirmed that in *Pleurotus eous* mushrooms UV exposure duration of 50 minutes showed highest conversion of ergosterol to vitamin D_2 in all the three forms (whole, sliced and powdered) with less or no significant reduction in quality and other nutritional composition. Whole and sliced mushroom treated with 1% acetic acid can retain all nutrients of mushroom, mainly vitamin D_2 during storage up to 6 days at refrigerated temperature. Powdered mushroom can be stored up to 1 month without loss of vitamin D_2 and other nutritional properties, however reports says that the powdered mushroom can be stored up to 3 months without losing the nutritional properties.

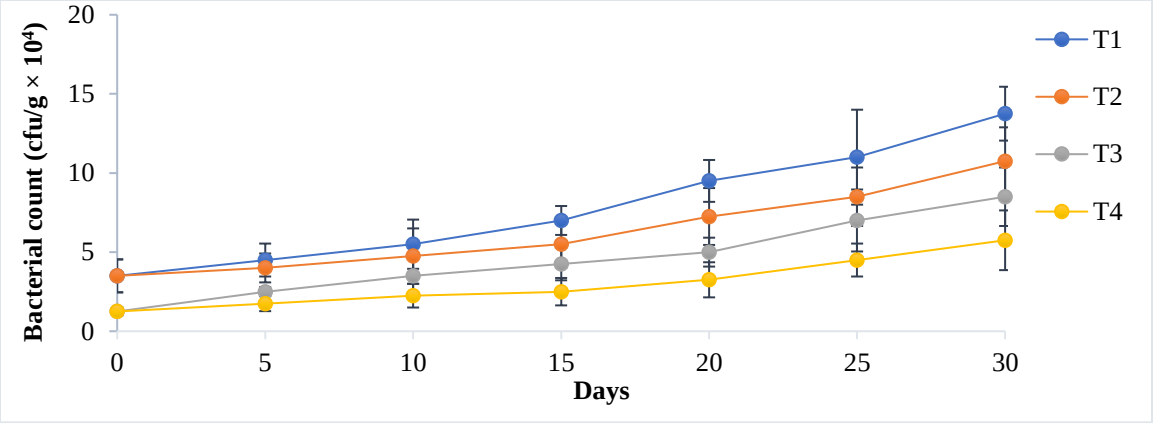


Fig. 17. Effect of UV radiation and storage temperature on change in bacterial count of powdered *Pleurotus eous* during storage period

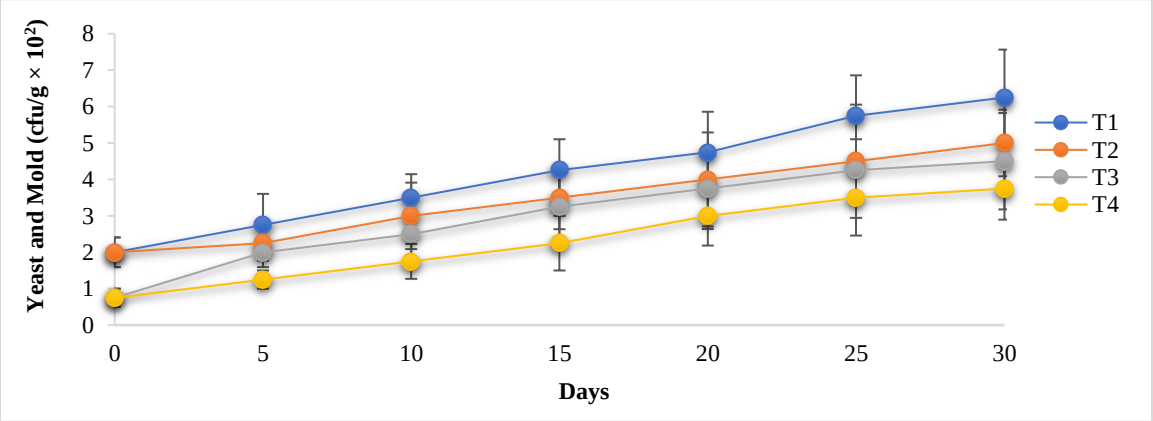


Fig. 18. Effect of UV radiation and storage temperature on change in yeast or mold count of powdered *Pleurotus eous* during storage period

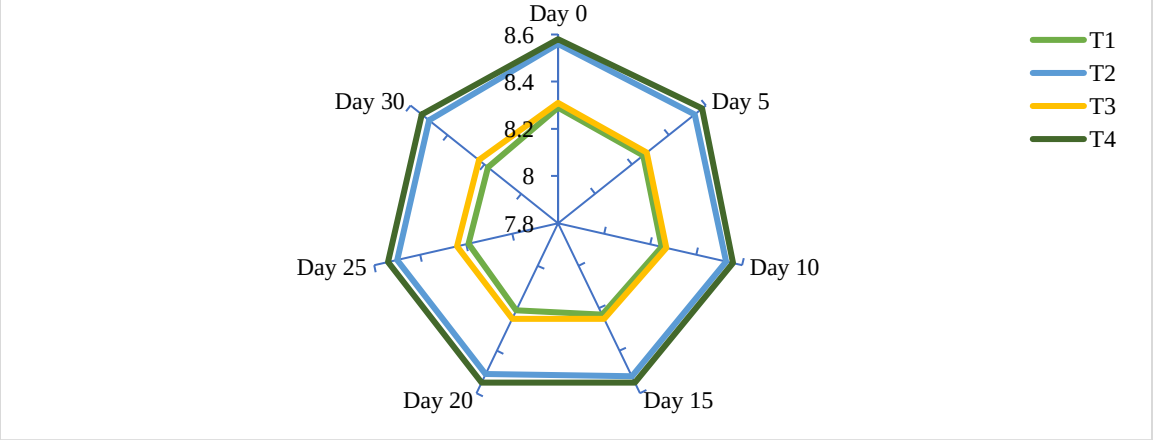


Fig. 19. Effect of UV radiation and storage temperature on overall acceptability based on sensory evaluation of powdered *Pleurotus eous* during storage period

Future line of work

1. Effect of types of UV on conversion of ergosterol to Vitamin D_2 in *Pleurotus eous* mushroom
2. Effect of distance of UV source and temperature on conversion of ergosterol to Vitamin D_2 in *Pleurotus eous* mushroom

SUMMARY

V SUMMARY

The world is currently facing a widespread deficiency of vitamin D, largely due to modern lifestyle practices and poor nutrition. Dietary sources of vitamin D are limited, like fatty fish, eggs, mushrooms, yoghurt, cod liver oil etc. Fortifying various foods can help to address this deficiency, among them mushrooms are one such nutrient-rich food. Oyster mushrooms, which have been consumed for thousands of years, are particularly appealing due to their nutritional profile. These mushrooms particularly oyster mushrooms contain ergosterol, a precursor to vitamin D, which can be converted into vitamin D through bond cleavage by exposure to the UV radiation. However, there was no literature available regarding fortification of vitamin D_2 to pink oyster mushroom (*Pleurotus eous*).

By considering these points, the present investigation entitled "Studies on enhancement of vitamin D_2 in oyster mushroom *(Pleurotus eous*) using UV radiation" in the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore during 2023-2024 and the results of the investigation were summarized here.

Fresh pink oyster mushrooms were harvested and are made into three different forms *viz*., whole, slice and powder form. The three forms were subjected to study the effect of UV radiation on vitamin D_2 content in oyster mushroom and the retention of vitamin D_2 content in UV radiation induced oyster mushroom during storage. The UV exposure of mushroom at different intervals of time leads to conversion of ergosterol to vitamin D_2. The three forms of mushroom were exposed to UV radiation at different time intervals, such as 0 minutes (T_1), 10 minutes (T_2), 20 minutes (T_3), 30 minutes (T_4), 40 minutes (T_5), 50 minutes (T_6), 60 minutes (T_7) and 70 minutes (T_8). Various parameters like physical (physiological loss in weight), biochemical (vitamin D_2, ergosterol, protein, carbohydrates, minerals,), microbiological analysis (for bacteria, yeast and mold) and visual quality assessment parameters (colour and texture) were analyzed.

Higher conversion of ergosterol to vitamin D_2 was observed in all the three forms of mushroom (whole: 21.95±0.45 µg/g f.w., slice: 41.37±0.85 µg/g f.w. and powder: 246.31±5.12 µg/g d.w.) when exposed to UV radiation for 50 minutes (T_6) compared other treatments and also there was a significant reduction in ergosterol content across all three

forms of mushrooms as the exposure duration increased. This may be due to the prolonged exposure to UV light, which causes the breakage of bond between C_9 and C_{10} in the B-ring of ergosterol, leading to the formation of pre-vitamin D_2. This pre-vitamin D_2 then undergoes a thermally induced rearrangement to form vitamin D_2.

There was no significant difference observed on protein content of all the three forms of mushroom upon irradiation of mushroom samples for varied time period (0 – 70 minutes). It was ranged from whole (2.77 % and 2.92 %), sliced (2.89 % and 2.98 %) and powdered mushroom (23.17 % and 25.38 %). This could be due to the stability of protein structure and not susceptible for the UV-induced photochemical reactions. Similarly, there was no change in the carbohydrates content in all the forms of mushroom on exposure to UV radiation for 0-70 minutes. This may be due to the carbohydrates are made up of sugar units, linked by glycosidic bonds. These bonds are relatively stable and do not undergo significant photochemical reactions when exposed to UV light.

The mineral content of mushrooms specifically phosphorus (P), potassium (K), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) upon exposure to UV radiation there was no significant change in all the three forms of mushroom. This could be due to their stable chemical structures, lack of photochemical reactivity and the absence of UV-sensitive functional groups.

The significant increase in weight loss from T_1 to T_8 for both whole and sliced mushrooms was observed. The maximum weight loss in treatment T_8 was due to the longer duration of exposure, which leads to decrease in moisture content, the UV light can disrupt the integrity of the cell walls, increasing permeability and causing water to evaporate from the mushroom tissues, which contributes to their weight loss.

The visual quality assessment indicated no significant difference in the color of mushrooms treated with UV radiation. However, a slight change in texture was observed in the whole and sliced mushrooms, likely due to UV-induced disruption of the cell wall, which impacted their firmness. There was no notable change in the color and texture of powdered mushrooms following UV treatment. The bacteria and yeast/mold population was reduced upon exposure to UV radiation in all the three forms of mushroom.

This experiment concludes that UV exposure duration of 50 minutes (T_6) showed highest conversion of ergosterol to vitamin D_2 in all the three forms (Whole, sliced and powdered) with less or no significant reduction in quality and other nutritional composition. Hence, UV exposure duration of 50 minutes (T_6) has been selected as a better duration of exposure and used further in storage study.

The influence of UV radiation and acetic acid preservative (1%) on the vitamin D_2 content of pink oyster mushroom was observed during storage for 6 days in whole and sliced forms. During the storage period, the vitamin D_2 content significantly decreased in all treatments, where T_3 (UV induced mushroom pre-treated with 1 % acetic acid and stored at 4°C) exhibiting the highest retention of vitamin D_2 in whole (18.38 ± 0.24 µg/g) and sliced (41.45±0.54 µg/g) forms of mushroom. The storage had no significant effect on the mushroom's ergosterol concentration for whole and sliced mushrooms.

The protein, carbohydrates and mineral content of the mushroom during the course of storage showed no significant difference among all the treatments for whole and sliced mushrooms. Further, the significant increase in physiological weight loss was observed in both whole and sliced mushroom, among which the maximum weight loss was observed in T_1 and minimum weight loss was observed in T_3. This difference may be attributed to UV-C irradiation, which effectively reduces weight loss by minimizing dehydration during storage.

The bacteria and yeast/mold population increased during the storage. The treatment T_3 (UV-induced whole mushrooms treated with 1% acetic acid and stored at 4°C) had the lowest bacterial (4.20×10^4 cfu/g) and yeast and mold growth (4.80×10^2 cfu/g) in whole form and the lowest bacterial (4.40×10^4 cfu/g) and yeast and mold growth (5.00×10^2 cfu/g) in slice form on the 6th day of storage.

The sensory evaluation (appearance, aroma, colour, texture and overall acceptability) was conducted with 21 semi-trained panel members from Department of Food Science and Nutrition, University of Agricultural Sciences, Bangalore based on the 9-point hedonic scale. It was found that UV induced mushroom treated with 1% acetic acid

and stored at refrigerator (T_3) had highest acceptability score of 6.28/9 (whole) and 6.48 (sliced) at 6th day of storage period.

In powdered mushroom, there was no significant change in the vitamin D_2 during storage. Instead, there a slight increase in vitamin D_2 of mushroom powder stored at room temperature T_1 (9.91 µg/g) and T_3 (265.40 µg/g) and there was a slight decrease in the ergosterol content of T_1 (3918 µg/g) and T_3 (3716 µg/100g). This may be due to additional ergosterol conversion to vitamin D_2 in mushrooms exposed to the artificial light during storage.

Over a period of storage upto 30 days, there was no significant change in protein content and carbohydrate content of T_2 (Powdered *Pleurotus eous* stored at 4°C) & T_4 (UV exposed powdered *Pleurotus eous* stored at 4°C). This is attributed to reduced microbial growth and enzymatic activity. There was no significant change in the all the minerals during storage, this may be attributed to the fact that many minerals do not readily react with moisture, light, or air, which helps maintain their stability during storage.

During the course of storage period, the microbial population was significantly increased in all treatments but the population was less in T_4 having the bacterial count of 5.75×10^4 cfu/g and yeast/mold count 3.72×10^2 cfu/g, this might be due to the antimicrobial property of acetic acid. It was found that UV induced powdered mushroom stored at refrigerator had high overall acceptability score of 8.58/9 at 30 days of storage period.

From this study, it can be concluded that UV-C treated mushroom represent an efficient and economic process for the production of dietary vitamin D_2. The highest yields of conversion of ergosterol into vitamin D_2 were obtained in all the three forms (whole, sliced and powdered) of mushrooms after UV-C irradiation for a duration of 50 minutes. Whole and sliced mushroom treated with 1% acetic acid after irradiation shown higher retention of vitamin D_2 along with the other nutrients up to 6 days, whereas powdered mushroom stored at refrigerator showed maximum retention of vitamin D_2 without significant loss in other nutritional properties up to 30 days of storage.

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APPENDICES

APPENDICES

1. Plate Count Agar (PCA)

Components	g/l
Tryptone	5 g
Yeast extract	2.5 g
Glucose	1 g
Agar	15 g
Distilled water	1000 ml

2. Potato Dextrose Agar (PDA)

Components	g/l
Potato dextrose broth (HiMedia)	24
Agar-agar	20
Distilled water	1000 ml

SENSORY EVALUATION SCORECARD

Name: **Date:**

Product Name:

Instruction:

- Please evaluate each of the following samples using scoring system given below.
- Write the preferred number score in the column as per evaluation.
- Rinse your mouth in between evaluating each sample.

Sample	Appearance	Texture	Colour	Aroma	Overall acceptability
T1					
T2					
T3					
T4					

Scoring system: 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely

Remarks: **Signature**

SENSORY EVALUATION SCORECARD

Name: **Date:**

Product Name:

Instruction:

- Please evaluate each of the following samples using scoring system given below.
- Write the preferred number score in the column as per evaluation.
- Rinse your mouth in between evaluating each sample.

Sample	Appearance	Texture	Colour	Aroma	Overall acceptability
T1					
T2					
T3					

Scoring system: 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely

Remarks: **Signature**