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Effect of UV Radiations on Vitamin D_2 Content and Nutritional Composition of Button (*Agaricus bisporus*) and Oyster (*Pleurotus florida*) Mushrooms

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Abstract: Button (*Agaricus bisporus*) and oyster (*Pleurotus florida*) mushrooms irradiated with ultraviolet (UV) radiations (UV-A, UV-B and UV-C) at a distance of 30, 45 and 60cm and with sun rays for 10, 20 and 30min followed by freeze drying resulted in an enormous increase in vitamin D_2 content. The irradiation dose of treating mushrooms for 30 min with UV-A, UV-B and UV-C was 17.9, 21.5 and 15.7 kJ/m^2, respectively. Irradiation of mushrooms with UV-B rays at a distance of 60 cm for 30 min resulted in the maximum spike in vitamin D_2 content with 228 and 141 folds increase in button mushrooms and oyster mushrooms, respectively. Freeze dried UV-B treated mushroom powder were significantly high in *in-vitro* protein digestibility and total phenols. The supplementation of UV treated button and oyster mushroom powder in traditional food recipe led to a significant increase in vitamin D_2 content, protein, ash, fibre and minerals like iron, copper, phosphorus, potassium, zinc and selenium, total phenol and *in- vitro* protein digestibility as compared to the control (without mushroom powder). There was retention in vitamin D_2 in the mushroom powder after incorporation in the food product. This vitamin-D_2 enriched mushroom powder can be extensively used as a food based approach to combat the problem of vitamin-D deficiency and to boost immunity. Mushroom powder and its supplemented food product stored in air tight glass containers for 5 months had negligible microbial growth.

Keywords: Button and Oyster mushrooms, Vitamin D_2 UV radiation, *in-vitro* protein digestibility, Total phenols

Vitamin D, the sunshine vitamin, has an important role in calcium and phosphorus metabolism. The prevalence of vitamin D deficiency ranges from 40% to 99%, with most of the studies reporting a prevalence of 80-90% and the prevalence is high all the age groups (Gupta and Gupta 2014). The main reason for this deficiency could be lack of sunlight exposure due to increased indoor lifestyle, increased pollution and reduced intake of vitamin D containing food. Vitamin D is present in two forms D_2 and D_3. Vitamin D_3 is mainly found in animal foods like eggs and fish whereas D_2 can be found in plant based sources (Barnkob et al 2016). Vitamin D may also be obtained from animal based food sources and dietary supplements (Elangovan et al 2017).Vitamin D is mostly present in the animal foods but the majority of the population in India is vegetarian so they are just left with a few options. Mushrooms being the only vegetarian food which contains Vitamin-D and this quantity can be enhanced by exposing the mushrooms to UV radiations and sunlight. Mushrooms are exactly similar to the skin of a human, can produce vitamin D on exposure to UV rays. A series of photochemical reactions occurring during the exposure process that leads to the conversion of ergosterol present on the mushroom surface to ergocalciferol (Vitamin D_2). This amount is quite high as compared to the amount of vitamin D present in the fortified foods. As fortified milk (100 g) has approximately 37 IU of vitamin D, whereas there could be an average amount of 19,000 IU of vitamin D in mushrooms (100g) irradiated with a dose of 15 KJ/ m^2 of UV-B (Aborhyem et al 2020). There is high prevalence of vitamin D deficiency all over the world and the synthetic supplements of vitamin D may not be easily available during this pandemic situation, so it is important to find out some locally available foods that are cost effective, natural, rich source of vitamin D and other macro and micro-nutrients, further would help to promote community health (Panarese and Shahini 2020).

Mushrooms are considered as a super food with huge benefits. Treatment of mushrooms with UV rays enhances their nutritional parameters (vitamin D_2 content, total phenols, *in-vitro* protein digestibility and fatty acid composition) for which Indian mushroom varieties need to be explored. Consumption of mushrooms exhibit great health benefits such as anti-inflammatory, antioxidant, antimicrobial, antitumor, hypoglycemic and antihypertensive (Carocho and Ferreira 2013a, Carocho and Ferreira 2013a, Alves et al

2013, Taofiq et al 2016). Since there is a glut production of mushrooms in winter season and it is a highly perishable crop due to high moisture content, so there is a need for value addition at commercial level. Processing of mushrooms into powder form will not only make them available throughout the year for better revenue generation but also provide a nutrient dense food for better nutritional security. The objective of this research was to explore the most suitable protocol to maximize the vitamin D_2 content in button and oyster mushrooms(both the varieties are widely consumed in an Indian scenario) using different UV rays in terms of wavelengths, intensity, distance, time duration of exposing these mushrooms to the UV rays. Then the UV treated mushrooms were further processed into powder to enhance their shelf life and incorporated in the Indian traditional recipe (*panjiri)* that would act as a food vehicle for improving the nutritional status of vitamin D deficient population.

MATERIAL AND METHODS

Raw material for experimentation: The samples of untreated fresh post-harvest button mushrooms (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus florida*) were procured from the Department of Microbiology, College of Basic Sciences, PAU, Ludhiana. Mushrooms of medium size used for experiment were harvested in morning time and transferred for processing in sealed containers in order to prevent the exposure to light before the irradiation to UV light. The moisture content of both the varieties of mushrooms were adjusted to around 80% by vacuum drying at 25° C before the start of the experiment for the maximum vitamin D_2 formation. For each treatment 100±15 gm of the mushroom sample was taken. Fresh button mushroom and oyster mushroom were treated in triplicate with different ultraviolet (UV) rays(UV-A, UV-B and UV-C) using different distances (30, 45 and 60 cm) from the source of irradiation and for different time durations (10, 20 and 30 min) in an irradiation chamber thus making a total of 162 samples.

Effect of duration and distance of UV rays on ergosterol conversion to Vitamin D_2: Fresh Button mushroom (*Agaricus bisporus*) and Oyster mushroom (*Pleurotus florida*) with different ultraviolet (UV) rays (UV-A, UV-B and UV-C) were treated in an irradiation chamber (made under the guidance of National Research Centre for Mushroom (NRCM), Solan). The dimensions of the chamber were 100 × 75 × 60 cm. Since UV rays does not pass through the PVC, all six surfaces of the chamber were covered by PVC sheets. The UV bulb were attached to a holder on the top center of the chamber, which has three removable internal stainless steel trays. The chamber allowed adjusting the distance between the tray and UV light source at 30, 45 and 60 cm. Medium sized mushrooms were sliced longitudinally and placed in the chamber with their gills upwards towards the radiation source for UV treatment for different time duration and at different distance. The gills side of the mushrooms were irradiated for 10, 20 and 30 min with ultra violet lights: UV-A bulb (wave length 315-400 nm , Philips TLD-100 watt with the intensity at 30 cm of 9.8 W/m^2), UV-B bulb (wave length 280-315 nm, Philips TL-100 watt/01 with intensity at 30cm of 11.5 W/m^2) and UV-C bulb (wave length 100-280nm, G18T8 units with intensity at 30 cm of 8.3 W/m^2) . Irradiated mushrooms were stored at -20ºC for 24 hrs. Mushrooms treated with UV-A, UV-B and UV-C had 0.59, 0.70, and 0.50 kJ/m^2/min of the irradiation doses rate and the doses of calculated radiation after 30 min of irradiation were 17.9, 21.5 and 15.7 kJ/m^2, respectively. The UV source showed a stable intensity and spectral distribution over the entire period of 30 min. During all steps of preparation, the mushroom samples were not exposed to incidental ultraviolet light. Mushroom samples were freeze dried at -40°C for 36-48hrs and pulverization into fine powder and stored in tight sealed aluminum coated poly bags. Non-irradiated mushrooms were freeze dried as a control. Nutritional evaluation of treated button and oyster mushroom powder was done which included fatty acid composition, total phenols, *in vitro* protein digestibility and vitamin-D_2 content. The traditional Indian food product supplemented with treated mushroom powder was analyzed for proximate composition, mineral content, total phenols, *in vitro* protein digestibility and vitamin D content.

Nutritional and Biochemical Analysis of UV Treated Mushroom Powder and its Supplemented Product

Vitamin D_2 estimation: AOAC 2002.05 after the addition of an internal standard (vitamin D_2) and basic hydrolysis, vitamin D_3 was extracted with *n*- heptane. The fraction that contains vitamin D_2/D_3 was separated by preparative normal-phase liquid chromatography (LC). After evaporation and dilution in acetonitrile-methanol, vitamin D_2 was determined by reversed-phase LC with UV detection at 265nm. A separate test portion was analyzed in parallel to confirm the absence of endogenous vitamin D_2 (AOAC 2012). Vitamin D_2 was qualified using formula given below:

$$\text{Quantity of vitamin } D_2\ (\mu g/g) = \frac{\text{Std concentration} \times \text{Area of sample} \times \text{Final volume} \times \text{Dilution factor}}{\text{Area of standard} \times \text{Weight of sample}}$$

Proximate composition: The highly acceptable experimental products along with their control samples were dried in hot air oven and milled with a grinder (Philips Grinder HL 1631/00) until a homogenous fine powder was obtained and were analyzed for proximate composition (moisture,

protein, fat, ash, carbohydrates and energy) using standard AOAC method (AOAC 2010)

Mineral estimation: The minerals namely iron, copper, phosphorus, potassium, zinc and selenium were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES) method using ICP optical emission spectrophotometer (ICP-OES Optima 2100 DV) after wet digestion (AOAC 2012).

Total phenolic content: For estimation of TPC methanol extract of samples were used. Estimation of TPC was conducted by Folin-Ciocalteu method (Mathur and Vijayvergia 2017). The total phenols in the sample were expressed as mg GAE (Gallic Acid Equivalent)/100g of dry weight.

Fatty acid composition (**Appleqvist 1968):** Fatty acid composition of food samples was estimated using gas liquid chromatography (GLC). The EZ chrome elite software was used to compute the fatty acid composition.

***In-vitro* protein digestibility:** Dry sample (0.5g) was taken in 250ml conical flask and added 50ml of pepsin solution Incubation at 37°C for 24 hrs was done. The residue was analyzed for N_2 content by macro- kjeldahl method. The digestibility coefficient was determined by subtracting the residue protein from the initial protein on the basis of 100g of sample (Singh et al 1989).

Shelf life evaluation: UV treated mushroom powders were stored and the developed indigenous products for a period of 5 months in a different packaging materials such as ziplock polythelene bags, glass container and plastic container at room temperature and further evaluated for shelf life (Thatcher and Clark 1968) for the presence of bacterial and yeast/ mold growth.

Statistical estimation: Multivariate analysis was used to study the effect of distance and time of exposure to different UV radiations on vitamin D_2 content. Independent sample t-test was applied for the comparison of nutritional parameters-proximate composition, mineral content, fatty acid composition, total phenols and *in vitro* protein digestibility between control sample and experimental sample by using computer software JMP 10.0.1.

RESULTS AND DISCUSSION

Formation of Vitamin D_2 in button and oyster mushroom: There was a significant effect of UV rays, distance and time interaction, on vitamin D_2 content of button mushroom when treated with different UV rays (Table 1). The vitamin-D_2 content of button mushroom treated with UV-A, UV-B, UV-C and sun rays was significantly higher at 7, 228, 43 and 59 folds in comparison to the control (Untreated button mushroom) when exposed at maximum distance of 60cm for maximum time duration at 30 min. The significant increase in vitamin D_2 content of *A. biporus* irradiated with UV-B rays is reported by earlier scientists (Simon et al 2011, Urbain and Jakobsen 2015, Bilbao- Sainz et al 2017). Exposure of fresh mushrooms to different wavelengths of UV radiations after harvesting lead to a tremendous increase in vitamin D_2 content reaching upto 40 g/g dried mass (ca 320 g/100 g FW) (Urbain and Jakobsen 2015, Koyyalamudi et al 2009, Urbain et al 2016). Ko et al (2008) reported UV-B radiation as the most effective wavelength to stimulate the production of vitamin D_2 in mushrooms (280-315 nm). Contrary to the results reported in the present research some researchers have reported UV-A 315-400 nm (Koyyalamudi et al 2009) and UV-C (<280 nm) radiations (Urbain et al 2016, Ko et al 2008) as a potential wavelength to enhance the vitamin D_2 content in mushrooms. There was a significant effect of UV rays, distance and time interaction, on vitamin D_2 content of oyster mushroom when treated with different UV rays (Table 2). For oyster mushroom exposed to all three UV rays such as UV-A, UV-B and UV-C rays, Vitamin-D_2 content was significantly increased by 1.19, 141 and 14 folds at the maximum distance of 60 cm for maximum time duration at 30min as compared to control (untreated oyster mushroom).Oyster mushroom when exposed to sun rays for 30 min, 10 folds increase in vitamin-D_2 was observed as compared to control oyster mushrooms. Thus UV-B rays significantly increased vitamin-D_2 content of oyster mushroom as compared to other treatments. Various researchers have emphasized the use of different UV

Table 1. Comparison of vitamin D_2 (µg/100g) content of button mushroom treated with various UV rays

Distance (cm)	Time (min)		
	10	20	30
Button mushroom treated with UV-A Rays			
30	21.60 ± 0.44	24.10 ± 0.57	31.87 ± 0.19
45	23.55 ± 0.18	25.52 ± 0.32	33.87 ± 0.45
60	26.70 ± 0.41	29.01 ± 0.54	36.57 ± 1.04
Button mushroom treated with UV-B Rays			
30	505.88 ± 5.12	730.6 ± 7.11	774.66 ± 6.29
45	796.45 ± 6.39	844.56 ± 2.71	886.73 ± 6.54
60	915.82 ± 3.71	1146.49 ± 4.01	1250.13 ± 1.05
Button mushroom treated with UV-C Rays			
30	111.21±0.62	131.38 ±1.08	157.69 ±2.20
45	148.09 ±2.30	182.13 ±1.45	202.68 ±2.09
60	223.15 ±2.63	232.30 ±2.82	237.88 ±2.65

Values are given in mean ± SD
Vitamin D_2 content of control (un-irradiated button mushrooms) was 5.48 ± 0.34 µg/100g

radiations in increasing the vitamin D_2 content of oyster mushrooms. *Pleurotus ostreatus,* the most common species of *Pleurotus* genus (oyster mushroom) reported the conversion of ergosterol to active form of vitamin D_2 on irradiation with UV-A rays (Ko et al 2008). The enhancement in vitamin D_2 on exposure to UV-B rays was reported (Banlangsawan and Sanoamuang 2016) and with UV-C rays (Slawinska et al 2016). Thus UV-B rays significantly increased vitamin-D_2 content of both Button and Oyster Mushroom as compared to other treatments and this UV-B treated button and oyster mushroom powders were further supplemented in various traditional Indian food recipe like *panjiri* at different 10 and 15% levels, respectively.

Table 2. Comparison of vitamin D_2 (µg/100g) content of oyster mushroom treated with various UV rays

Distance (cm)	Time (min)		
	10	20	30
Oyster mushroom treated with UV-A Rays			
30	88.38 ±1.08	91.30 ±0.65	94.67 ±1.37
45	90.09 ± 0.54	93.85 ± 2.84	97.05±1.58
60	93.36 ±2.19	95.34 ±0.61	99.54 ±0.58
Oyster mushroom treated with UV-B Rays			
30	4522.23 ±0.97	7753.68±3.96	9375.69 ±3.01
45	5453.67±2.33	8103.01±2.76	10305.14±3.84
60	6721.90±1.22	8956.09±2.45	11687.3±2.05
Oyster mushroom treated with UV-C Rays			
30	1133.87 ±1.11	1147.97 ±1.88	1176.63 ±2.97
45	1148.19 ± 2.58	1155.53 ± 3.55	1182.45 ±2.94
60	1168.66 ±2.52	1175.11 ± 3.51	1192.30 ± 1.50

Vitamin D content of control (un-radiated oyster mushrooms) was 83.17 ± 2.02 µg/100g

Nutritional Evaluation UV-B treated Button and Oyster Mushroom Powder Supplemented Products

Proximate composition: The incorporation of UV-B treated button and oyster mushroom in the traditional Indian recipe like *panjiri* significantly improved the nutritional composition in terms of protein, fat, ash and fibre content as compare to control p*anjiri* prepared by using only whole wheat flour (Table 3). The vitamin D_2 enriched mushroom powder when incorporated in *panjiri* was further analyzed for its Vitamin D content. The vitamin D_2 content of the mushroom powder after incorporation in the food product retained as such with no deterioration. This enriched quality of vitamin D_2 is retained in mushroom powder supplemented food products. The vitamin D_2 content of *panjiri* supplemented with Vitamin D_2 enriched button mushroom powder at 10% of supplementation was 125ug/100 mg and with oyster mushroom powder at 15% was 1753µg/100mg. In case of control *panjiri* with no mushroom powder had zero vitamin D_2 content. Thus the proximate composition and vitamin D_2 content of UV-B treated button and oyster mushroom supplemented *panjiri* was enhanced which can further help to improve the nutritional status of the population by incorporating these mushroom powders in the traditional food recipes. The results of the present study are in accordance with the results of the previous researches which reported an enhancement of nutritional composition of traditional products by the addition of mushroom powder (Ishara et al 2018). The addition of button and oyster mushroom powder significantly enhanced the overall nutritional composition of *panjiri* in terms of protein, ash, fibre and fat content.

Table 3. Proximate composition UV-B treated button and oyster mushroom powder supplemented products (on dry weight basis)

Panjiri	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)	Energy (Kcal)
Panjiri supplemented with treated button mushroom powder						
Control	4.12±0.29	26.55±1.21	0.95±0.12	0.79±0.13	64.67±2.01	514.10±3.54
Experimental (10% TBMP)	6.69±0.72	27.29±1.07	1.83±0.10	2.86±0.22	58.47±0.77	510.22±1.77
|t|-Value	5.727	0.789	10.041	13.976	4.995	1.701
p-Value	0.005	0.474	0.001	<0.0001	0.008	0.164
Panjiri supplemented with treated oyster mushroom powder						
Control	4.12±0.29	26.55±1.21	0.95±0.12	0.79±0.13	64.67±2.01	514.10±3.54
Experimental (15% TOMP)	5.08±0.23	26.95±1.16	1.30±0.10	3.09±0.18	61.05±1.06	507.56±6.55
|t|-Value	4.521	0.418	3.880	17.799	2.762	1.522
p-Value	0.011	0.697	0.018	<0.0001	0.051	0.203

CBMP- Control Button Mushroom Powder (un-radiated)
TBMP- UV-B Treated Button Mushroom Powder at the distance of 60 cm for 30 min
COMP- Control Oyster Mushroom Powder
TOMP- UV-B Treated Oyster Mushroom Powder at the distance of 60 cm for 30 min
t-values are absolute values

Mineral content (dry weight basis): There was a significant increase in the mineral content (iron, copper, phosphorus, potassium, zinc and selenium) of *panjiri* supplemented with 10% of UV-B treated Button and 15% UV B treated oyster mushroom powder as compared to control *panjiri* (Table 4). These results are in accordance with the results reported in previous years where *panjiri* supplemented with other functional foods led to a better nutritional composition in terms of its mineral composition (Kaur and Sharma 2017, Dhanesh et al 2018).

Nutritional Composition of UV-B Treated Mutton and Oyster Mushrooms

Fatty Acid composition: There was a significant decrease in linoleic acid in UV treated button and oyster mushrooms as compared to control mushrooms (Table 5). In oyster mushrooms, there was a significant increase in oleic acid after UV treatment. Saturated fatty acid also significantly increased in oyster mushrooms after UV treatment, though it decreased non-significantly in button mushroom. The variation in the fatty acid composition of treated button and oyster mushroom powder might depend on the cellular mechanism of lipid metabolism. Acetyl-CoA carboxylase is an enzyme that plays a key role in fatty acid synthesis which is dependent upon the availability of ATP's (adenosine triphosphate). Biosynthesis of PUFA requires large amount of ATP's than SFA's and MUFA's production. UV exposure may decrease the availability of ATP's for the synthesis of fatty acids which can cause decrease in linoleic fatty acid composition of UV treated button and oyster Mushroom Powder. Guihéneuf et al (2010) also observed that UV treatment led to reduction in PUFAs such as 20% in EPA and 16% in DHA in two marine microalgae *Pavlova lutheri* (*Pavlovophyceae*) and *Odontella aurita* (*Bacillariophyceae).*

Total phenols: There was a significant increase in total phenols in mushrooms after UV B radiations (Table 6). UV-B exposure causes the abiotic stress in plants that produces two natural enzymes, polyphony alanine ammonia-lyase and chalcone synthase as a mechanism to adapt the stress

Table 4. Mineral content of UV-B treated button and oyster mushroom powder supplemented products (on dry weight basis)

Panjiri	Iron (mg/100g)	Copper (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Zinc (mg/100g)	Selenium (mg/100g)
Mineral content of panjiri supplemented with UV-B treated button mushroom powder						
Control	1.53±0.06	1.82±0.12	396.01±1.09	339.53±1.26	0.10±0.02	0.04±0.01
Experimental (10% TBMP)	4.21±0.11	3.76±0.15	636.76±0.63	673.47±0.67	1.62±0.11	0.89±0.03
|t|-Value	20.590	10.132	191.370	234.546	14.021	26.849
p-Value	<0.0001	0.001	<0.0001	<0.0001	<0.0001	<0.0001
Mineral content of Panjiri supplemented with UV-B treated oyster mushroom powder						
Control	1.53±0.06	1.82±0.12	396.01±1.09	339.53±1.26	0.10±0.02	0.04±0.01
Experimental (15% TOMP)	5.29±0.16	4.03±0.02	703.95±2.20	710.08±0.92	2.55±0.21	0.23±0.02
|t|-Value	21.370	18.271	125.586	238.432	11.590	9.848
P-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001

Values are mean± SE, See Table 3 for details

Table 5. Fatty acid composition of UV treated button and oyster mushroom powder (on dry weight basis)

Treatment	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2,ω-6)
Fatty acid composition of button mushroom powder				
CBMP	14.55 ±0.11	5.68 ±0.06	4.66 ±0.19	76.00 ±0.14
TBMP	14.73 ±0.03	5.80 ±0.06	3.90 ±0.35	74.78 ±0.10
|t|-Value	2.602	2.376	3.372	12.414
p-Value	0.060	0.076	0.028	<0.0001
Fatty acid composition of oyster mushroom powder				
COMP	16.60±0.09	4.92±0.33	25.71±0.01	52.85±0.27
TOMP	17.09±0.27	6.75±0.03	28.96±0.04	47.16±0.25
|t|-Value	3.083	9.554	137.60	26.934
p-Value	0.037	0.001	<0.0001	<0.0001

See Table 3 for details

conditions which further synthesize the phenolics and chemical compounds. Thus this led to the growth of phenolic compounds in the plants (Liu et al 2011). Similar results, regarding increase in total phenolic content and other antioxidant properties of medicinal caterpillar fungus *Cordyceps militaris* on exposure to UV-B rays was reported by Huang et al (2015).

***In-vitro* protein digestibility:** There was a significant increase in *in-vitro* protein digestibility of button and oyster mushrooms after exposure to UV-B radiations (Table 6). Previous studies have also reported an effect of irradiation on protein structure and digestibility. Li et al (2020) reported that due to irradiation solubility of proteins increased, increased the polyphenols and these directly affects the protein digestibility. Further freeze drying is an efficient processing method which retained the nutrients with least losses.

Shelf life of UV-B treated mushroom powder and its supplemented products: UV-B treated mushroom powder and its supplemented indigenous food product (*panjiri*) were stored in three different containers: zip lock polyethylene bags, plastic containers and glass containers for a period of 5 months at room temperature and its microbial analysis was done. Initially no microbial growth was observed in stored material till 90th day. The bacterial growth was initiated in stored mushroom powder at 105th day of storage period and with the increase in storage period the bacterial count increased consistently. The bacterial count of the stored button mushroom powder in glass container was significantly lower at $0.33x10^2$cfu/g in comparison of Zip lock Polyethylene Bag and Plastic container after 5 months of storage. There was significant effect of packaging material and time on bacteria count of treated button mushroom powder. The yeast and mold count of treated button mushroom powder stored in different containers was assessed for 150 days of storage period. Initially no yeast and mold growth was observed till 105th day of storage period. The yeast and mold count was initiated at 120th day of storage period. After storage period of 150 days the yeast and mold count was higher in zip lock polyethylene bag packaging ($0.85x10^2$cfu/g) as compared to plastic container and glass container in UV treated button mushroom powder. There was significant effect of time on yeast and mold count. Thus the glass container is considered to be a better storage container as compared to other containers in terms of its yeast and mold count.

Similar results were observed for UV-B treated oyster mushroom powder stored in different containers. The bacterial count of zip lock polyethylene bag packaging was higher at $1.33x10^2$cfu/g than plastic container and glass container containing UV treated oyster mushroom powder. There was a significant effect of time on bacteria count. There was a significant increase of yeast and mold count during storage period. In UV treated oyster mushroom powder the fungi count was observed higher in zip lock polyethylene bag ($1.18x10^2$cfu/g) as comparison of plastic container and glass container. There was a significant effect of packaging material and time on yeast and mold count of treated oyster mushroom powder. Thus can conclude that Glass container is the good material for storage as compared to zip lock polyethylene bag and plastic container as the fungi growth was found significantly higher in zip lock polyethylene bag followed by plastic containers. Thus, UV-B treated mushroom powder and its supplemented products could be safely stored in air tight glass containers at room temperature for a period of 5 months.

Table 6. Total phenols and *in vitro* protein digestibility in UV treated button and oyster mushroom powders and their supplemented products (on dry weight basis)

Treatment	Total phenols (mg/100g)	*In-vitro* protein digestibility
CBMP	386.67±0.64	74.33±0.57
TBMP	496.67±0.64	84.25±0.87
|t|-Value	121.250	17.775
p-Value	<0.0001	<0.0001
COMP	364.07±0.98	73.32±2.68
TOMP	421.11±0.64	83.27±0.87
|t|-Value	48.699	6.127
p-Value	<0.0001	<0.0001
Control *Panjiri*	58.89±0.64	75.94±1.05
Experimental *Panjiri* (10%TBMP)	144.44±0.64	79.75±0.58
|t|-Value	94.305	5.495
p-Value	<0.0001	0.005
Control *Panjiri*	58.89±0.64	74.33±0.57
Experimental *Panjiri* (15%TOMP)	91.11±0.64	83.27±0.78
|t|-Value	35.518	4.102
p-Value	<0.0001	0.015

See Table 3 for details

CONCLUSIONS

The study concluded that out of the three UV wavelengths used to irradiate button and oyster mushrooms, UV-B rays treatment at a distance of 60 cm for a duration of 30 min resulted in an enormous spike in Vitamin D_2 content in mushrooms. Further this enriched quality of vitamin D was retained in mushroom powder supplemented food products. The UV treatment of mushrooms enhanced its nutritional composition in terms of total phenols, *in vitro* protein quality

and fatty acid composition. There was a significant increase in protein, fibre and ash content, mineral like iron, copper, phosphorus, potassium, zinc and selenium; total phenols , *in vitro* protein digestibility and Vitamin D content in UV treated mushroom powder supplemented indigenous food products. Vitamin-D_2 enriched mushroom powder can be extensively used as a food based approach to combat the problem of vitamin-D deficiency, address micro nutrient deficiencies and help in building up the immunity in this pandemic situation.

REFERENCES

Aborhyem S, Hamza D and Agamy N 2020. Vitamin D content in some food items sold in Egyptian markets. *Canadian Journal of Clinical Nutrition* **8**: 62-77.

Alves MJ, Ferreira ICFR, Dias J, Teixeira V, Martins A and Pintado M 2013. A review on antimicrobial activity of mushroom extracts and isolated compounds. *Planta Medica* **78**: 1707-1718.

AOAC 2010. *International Official methods of Analysis,* 18th edition, Association of Official Analytical Chemists, Washington DC

AOAC 2012. *Official methods of analysis.* 19th edition, AOAC International, Rockville, MD. Method 2002.05. www.eoma.aoac.org

Appleqvist LA 1968. Rapid methods of lipid extractions and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipids contaminants. *Arkiv Foe Kemi Royal Swedish Academy of Science* **28**: 551-570.

Banlangsawan N and Sanoamuang N 2016. Effect of UV-B irradiation on contents of ergosterol, vitamin D_2, vitamin B_1 and vitamin B_2 in Thai edible mushrooms. *Chiang Mai Journal of Science* **4**: 45-53.

Barnkob LL, Argyraki A, Petersen PM and Jakobsen J 2016. Investigation of the effect of UV-LED exposure conditions on the production of Vitamin D in pig skin. *Food Chemistry* **212**: 386-391.

Bilbao-Sainz C, Chiou BS, Williams T, Wood D, Du WX, Sedej I and McHugh T 2017. Vitamin D-fortified chitosan films from mushroom waste. *Carbohydrate Polymers* **167**: 97-104.

Carocho M and Ferreira ICFR 2013a. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology* **51**: 15-25.

Carocho M and Ferreira ICFR 2013b. The role of phenolic compounds in the fight against cancer: A review. *Anti-cancer Agents in Medicinal Chemistry* **13**: 1236-1258.

Dhanesh TB, Kochhar A and Javed M 2018. Effect of supplementation of partially defatted peanut cake flour and dehydrated spinach leaves powder on the nutritional quality of Indian seasonal sweets. *International Journal Current Microbiology Applied Science* **7**: 384-392.

Elangovan H, Chahal S and Gunton JE 2017. Vitamin D in liver disease: Current evidence and potential directions. *Biochimica et Biophysica Acta* **1863**: 907-916.

Gupta R and Gupta A 2014. Vitamin D Deficiency in India: Prevalence, causalities and interventions. *Nutrients* **6**: 729-775.

Guan W, Zhang J, Yan R, Shao S, Zhou T, Lei J and Wang Z 2016. Effects of UV-Ctreatment and cold storage on ergosterol and vitamin D2 contents in different parts of white and brown mushroom (*Agaricus bisporus*). *Food Chemistry* **210**: 129-134.

Guihéneuf F, Fouqueray M, Mimouni V, Ulmann L, Jacquette B and Tremblin G 2010. Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae *Pavlova lutheri* (*Pavlovophyceae*) and Odontella aurita (*Bacillariophyceae*). *Journal of Applied Phycology* **22**: 629-638

Huang G, Cai W and Xu B 2016. Vitamin D2, ergosterol, and vitamin B2 content in commercially dried mushrooms marketed in China and increased vitamin D2 content following UV-C irradiation. *International Journal Vitamins Nutrition Research* **1**: 1-10.

Huang SJ, Lin CP and Tsai SY 2015. Vitamin D_2 content and antioxidant properties of fruit body and mycelia of edible mushrooms by UV-B irradiation. *Journal Food Composition Analysis* **42**: 38-45.

Ishara JRM, Sila DN, Kenji GM and Buzera AK 2018. Nutritional and functional properties of mushroom (*Agaricus bisporus & Pleurotus ostreatus*) and their blends with maize flour. *American Journal Food Science and Technology* **6**: 33-41.

Jasinghe VJ and Perera CO 2005. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D2 by UV irradiation. *Food Chemistry* **92**: 541-546.

Kaur M and Sharma S 2017. Development and nutritional evaluation of pumpkin seed (*Cucurbita moschata*) supplemented products. *Food Science Research Journal* **8**: 310-318.

Ko JA, Lee BH, Lee JS and Park HJ 2008. Effect of UV-B exposure on the concentration of vitamin D2 in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus*). *Journal Agricultural Food Chemistry* **56**: 3671-3674.

Koyyalamudi SR, Jeong SC, Song CH, Cho KY and Pang G 2009. Vitamin D2 formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet irradiation. *Journal Agricultural Food Chemistry* **57**: 3351-3355.

Liu P, Kallio H and Yang B 2011. Phenolic compounds in hawthorn (*Crataegus grayana*) fruits and leaves and changes during fruit ripening. *Journal of Agricultural and Food Chemistry* **59**: 11141-11149.

Li Z, Chu S and Wang P 2020. Effects of irradiation treatment on protein structure and digestion characteristics of seed-watermelon (*Citrullus lanatus* var.) kernel protein. *Food Science Biotechnology* **29:** 1201-1211 .

Mathur R and Vijayvergia 2017. Determination of total flavonoid and phenol content in *Mimusops elengi* L. *International Journal of Pharmaceutical Sciences and Research* **8**: 5282-5285.

Panarese A and Shahini E 2020. Covid-19, and vitamin D. *Alimentary Pharmacology & Therapeutics* **51***:* 993-997.

Slawinska A, Fornal E, Radzki W, Skrzypczak K, Zalewska-Korona M, Michalak- Majewska M and Stachniuk A 2016. Study on Vitamin D2 stability in dried mushrooms during drying and storage. *Food Chemistry* **199**: 203-209.

Simon RR, Phillips KM, Horst RL and Munro IC 2011. Vitamin D mushrooms: Comparison of the composition of button mushrooms (*Agaricus bisporus*) treated postharvest with UVB Light or Sunlight. *Journal of Agric Food Chemistry* **59**: 8724-8732.

Singh U, Rao PV, Seetha R and Jambunathan R 1989. Nutrient loss due to scarification of pigeonpea (*Canjanus cajan L.*) cotyledons. *Journal of Food Science* **54**: 974-976.

Taofiq O, González-Paramás AM, Martins A, Barreiro MF and Ferreira ICFR 2016. Mushrooms extracts and compounds in cosmetics, cosmeceuticals and nutricosmetics: A review. *Industrial Crops and Products* **90**: 38-48.

Thatcher FS and Clark DS 1968. *Microorganism in foods: Their significance and methods of enumeration*. Canada: University of Toronto Press.

Urbain P and Jakobsen J 2015. Dose-response effect of sunlight on vitamin D_2 production in Agaricus bisporus mushrooms. *Journal of Agriculture Food Chemistry* **63**: 8156-8161.

Urbain P , Valverde J and Jakobsen J 2016. Impact on vitamin D2, vitamin D4 and agaritine in Agaricusbisporus mushrooms after artificial and natural solar UV light exposure. *Plant Food Human Nutrition* **71**: 314-321.

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