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RESEARCH ARTICLE

Enhancement of nutraceutical components of mushroom by uv exposure and extension of their shelf-life using edible coating material adopting online assessment of keeping quality by Magnetic Resonance Imaging (MRI) technique

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ABSTRACT

To enhance the nutraceutical value and preservation of mushrooms, this study focused on augmenting two widely consumed mushroom varieties in India namely the Button Mushroom (*Pleurotus ostreatus*) and Oyster Mushroom (*Agaricus bisporus*). These mushrooms were subjected to UV light exposure to evaluate their impact on phytochemical content, including phenolics, flavonoids, folic acid, and Vitamin D2. The percent increase in phenolic content after 120 minutes of UV treatment was 0.6-fold for *Agaricus bisporus* and 0.7-fold for *Pleurotus ostreatus*. Notably, *A. bisporus* exhibited a particularly high phenolic content of up to 13.5 mg per gram dry weight of mushroom after 120 minutes of UV exposure, in contrast to *P. ostreatus*, which displayed 8.7 mg per gram dry weight of mushroom under the same conditions. This study also revealed a threefold increase in flavonoid content in *Pleurotus ostreatus*, rising from 1.2 mg of quercetin/g in the control to 4.1 mg of quercetin/g after UV exposure for 120 minutes. Conversely, a sevenfold increase in quercetin content was observed in *Agaricus bisporus*, surging from 0.45 g (control) to 3.2 g following 120 minutes of UV exposure. The exposure of UV light for 60 minutes resulted in the highest absorbance of vitamin D2 at 0.81 OD in *Pleorotus osteatus* compared to 0.46 in *Agaricus bisporus*. Additionally, applying agar or gelatine coatings led to a shelf-life extension of up to 14 days under standard storage conditions. The use of MRI for quality determination of mushrooms by evaluating the lipid profile in the samples was reported for the first time. This study has shown a significant enhancement of nutraceutical components of mushrooms with special reference to phenolics, flavonoids, folic acid, and vitamin D2 for value addition, with a simple intervention of UV treatment. Moreover, the shelf life of mushrooms could be enhanced by agar and gelatin coating thereby extending the keeping quality. The use of MRI for evaluating the quality of mushrooms has also been the hallmark of this study. The practical utility of all the above findings has immense industrial application in the large-scale production of highly nutritive mushrooms.

Keywords: *Pleurotus ostreatus*; *Agaricus bisporus*; UV light; Agar coating; shelf life

1. Introduction

Edible mushrooms have garnered global acclaim and are increasingly popular due to their nutritional and medicinal benefits[1]. Roman et al.[2] reported that numerous mushroom species hold significant medicinal value, widely utilized in traditional medicine for a wide array of ailments. When exposed to sunlight, mushrooms naturally synthesize Vitamin D, potentially augmenting their nutritional value for enhanced calcium absorption and bone strength in humans[3]. Vitamin D is associated with various health advantages, including promoting healthy bones, preventing rickets and osteoporosis, and reducing the risk of heart disease, type 2 diabetes, hypertension, and colorectal cancer[4,5]. UV light has found application as a germicidal agent

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in water treatment and surface disinfection, owing to its ability to disrupt the DNA of microorganisms. Conversely, low doses of UV irradiation can induce favorable reactions in biological materials like fruits and vegetables, leading to an array of beneficial effects, such as prolonging shelf-life or increasing the content of health-promoting components[6]. UV radiation holds the potential for sanitizing fresh mushrooms, offering a non-chemical means of preserving mushroom quality and extending their postharvest life.

Magnetic Resonance Imaging (MRI) is a non-invasive scanning technique utilizing strong magnetic fields and radio waves to generate detailed internal tissue images. MRI serves as an invaluable tool for non-invasively diagnosing and monitoring liver function and iron levels[7]. Additionally, MRI has proven useful in assessing tissue hydration/dehydration status. In this study, MRI is employed for the first time to measure fat content in mushrooms, providing a non-invasive means of monitoring their keeping quality[8].

This research was designed to enhance the levels of Vitamin D, folic acid, phenolic compounds, flavonoids, and antioxidant properties in mushrooms through UV irradiation. Furthermore, the extension of shelf life in mushrooms using a polysaccharide-based emulsion was investigated to improve storage longevity. A novel approach involving MRI was employed to track the status of coated mushrooms, offering an innovative means of quality assessment. Overall the objective was to enhance the nutraceutical profile, keeping quality with the adoption of appropriate technologies of utility to the mushroom industry.

2. Materials and methods

2.1. Materials

All the chemicals and reagents used in this study were commercial grade.

2.2. Selection of samples

Button Mushroom (*Pleurotus ostreatus*) and oyster Mushroom (*Agaricus bisporus*) samples were obtained from Horticultural Producer's Cooperative Marketing and Processing Society Limited, Bangalore, Karnataka.

2.3. Exposure to UV radiation

Hundred grams of fresh mushrooms (*Agaricusbisporus* and *Pleurotusostreatus*) were cleaned and washed in double distilled water and blot dried prior to exposure to UV light at 253 nm in UV chamber for different time intervals such as 5, 15, 30, 45, 60 minutes respectively, at 25 ℃. They were analyzed for the nutraceutical and phytochemicals as given below. Controls received the same processing conditions except for being incubated in the dark.

2.4. Sample preparation

Every sample comprised of complete mushroom fruiting bodies (cap, gills, tubes, and stipes) of different sizes. The fruiting bodies of

mushrooms were cleaned and washed to remove any residual compost by using distilled water. These samples were cut into pieces and stored at −80 ℃. Then, it was ground into fine powder by using a blender and later stored in plastic bags in desiccators till analysis.

2.5. Extraction of components of mushroom for analytical procedures

Extraction was performed according to the method of Yang et al.[9]. Five grams of each of powdered sample of mushrooms were ground and extracted with 100 ml of absolute ethanol. The mixture of the homogenate and ethanol was placed in a conical flask (wrapped with aluminum foil) and agitated at 200 rpm with an orbital shaker for 1 hour at 25 ℃. The extract was then separated from the residue by filtration through sintered bottom filter. The remaining residue was re-extracted twice, and pooled. The residual solvent of ethanoic extracts was removed under reduced pressure at 40 ℃ using a rotary evaporator to dryness. For aqueous extraction, the aqueous extract was lyophilized using a freeze dryer. Both of the dried extracts were used directly for all analyses.

2.6. Determination of total phenolic content

The content of phenolics in the sample extracts was determined by using Folin-Ciocalteu assay, based on the procedure described by Olajire and Azeez[10]. One ml of sample from aqueous or ethanol extract was mixed with 1 ml of Folin- Ciocalteu's phenol reagent (1:9; Folin-Ciocalteu reagent: distilled water). After 5 min, 1 ml of 13% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min and its absorbance was read at 725 nm. A calibration curve was constructed with different concentrations of Gallic acid as standard. The results were expressed as mg of Gallic acid equivalents (GAE) per gram dry weight of mushrooms.

2.7. Determination of total flavonoid content

The $AlCl_3$ method was used for the determination of the total flavonoid content of the sample extracts according to the method described by Jagadish et al.[11] with some modifications. Ethanol extract (1.5 ml) was mixed with 5 ml of deionized distilled water (ddH_2 O) and 0.3 ml of 5% $NaNO_2$. After five min incubation at room temperature, 1.5 ml of 2% Aluminium trichloride ($AlCl_3$) solution was added. Two milliliters of 1M NaOH was added after the next 6 min. The mixture was vigorously shaken on an orbital shaker for 5 min at 200 rpm and the absorbance was read at 510 nm against a blank. Quercetin with different concentrations was used as a standard for constructing a standard curve. The results were expressed as flavonoid, quercetin equivalent in mg per gram dry weight of mushrooms.

2.8. Determination of folic acid

Folic acid was determined by the method of Matias[12]. The standard assay mixture was prepared by dissolving 1 mg of folic acid in a mixture of 1.0 ml of 4 M hydrochloric acid. Various aliquots of standard folic acid ranging from 20–100 ug were dispensed into 1.0 ml of 4 M hydrochloric acid and mixed with 1 ml of 1% (w/v) sodium nitrite, 1 ml of 1%(w/v) sulfamic acid and 1 ml of 1% (w/v) 3-aminophenol which resulted in an orange-yellow complex. The absorption of complexation was measured at 460 nm using UV- Visible spectrophotometer. The aliquots of aqueous extracts of control and UV-treated mushrooms were taken for analysis and processed in the manner described above. The absorption was compared with the standard curve prepared for folic acid. The content of folic acid in the sample was expressed as µg/ mg dry weight of mushrooms.

2.9. Analysis of Vitamin D_2

Spectrophotometric determination of vitamin D_2 was carried out by the method described by Saad et al.[12], where calciferol reacts with 1N hydrochloric acid in the presence of symmetrical tetrachloroethane to develop a greenish-yellow color with maximum absorption at 450 nm. Vitamin D_2 levels were proportional to the OD. Therefore, the absorption value was used as a criterion of relative Vitamin D_2 Levels among the treatments and controls as recommended by Hassan[13].

2.10. Coating of mushrooms

The effects of three edible polysaccharide coatings including Agar (3%), Gelatin (3%) and Carboxy Methyl Cellulose (2%) were investigated on the shelf life of mushrooms. Ascorbic acid 0.1% W/V was used for pre-treatment of mushrooms, for 5 minutes as antioxidant to prevent browning.

2.11. Microbial cell counts

Microbial population in coated mushrooms were evaluated after 14 days of coating for their quality assessment. Pour plate technique was used for determining microbial cell count as described by Olsen and Bakken[14].

2.12. Weight loss

The samples from each treatment were weighed during the study with an analytical weight balance. The results were expressed as percent weight loss[15].

2.13. Quality assessment by MRI scan

Magnetic resonance imaging (MRI) is an imaging modality that uses strong magnetic fields and radio waves to produce detailed images of the structure, function, and metabolism in tissues.MRI scan gives two types of maps—T_1 Map and T_2 Map. The T1 map gives the lipid profile and the T2 map gives the water profile[16]. MRI was used for the first time for scanning of Mushrooms to determine the changes in lipid content as a measure of quality of polysaccharide coated mushrooms.

2.14. Statistical analysis

Results obtained were the mean of three determinants. Analysis of variance was carried out on all data at $p < 0.05$ using one way ANOVA

3. Results and discusion

3.1. Enhancement of Phenolics in mushrooms upon UV exposure

The mushroom varieties were exposed to UV light for a specified time (0, 5, 10, 15, 30, 45, 60 and 120 minutes) followed by analytical studies. A significant increase in phenolic content was found with the increase in time of UV irradiation (**Table 1**). The percent increase in phenolic content after 120 minutes of UV treatment was 0.6-fold for *Agaricus bisporus* and 0.7-fold for *Pleurotus ostreatus*. Notably, *A. bisporus* exhibited a particularly high phenolic content of up to 13.5 mg per gram dry weight of mushroom after 120 minutes of UV exposure, in contrast to *P. ostreatus*, which displayed 8.7 mg per gram dry weight of mushroom under the same conditions. This result is in accordance with Yim et al.[17], who has also reported similar results of elevated phenolic levels in mushrooms upon UV treatment.

Table 1. Total phenolic content of UV irradiated mushrooms.

Time of UV irradiation (minutes)	**Total phenolic content in *Agaricusbisporus* (mg of GAE*/g DW)**	**Total phenolic content in *Pleurotus ostreatus* (mg of GAE*/g DW)**
0 (Control)	8.22 ± 0.107	5.1 ± 0.043
5	11.4 ± 0.28	6.2 ± 0.056
15	11.6 ± 0.012	6.93 ± 0.34
30	12.2 ± 0.24	7.1 ± 0.087
45	12.98 ± 0.003	7.69 ± 0.008
60	13.26 ± 0.05	8.32 ± 0.076
120	13.5 ± 0.93	8.7 ± 2.52

*Gallic acid equivalents (GAE) mg gallic acid per gram dry weight of mushrooms.

3.2. Enhancement of Flavonoid in UV irradiated mushrooms

Flavonoid content was relatively higher in *Pleurotus ostreatus* (1.2 ± 0.14 mg of Quercitin/g) compared with *Agaricus bisporus*in (0.45 ± 0.93) in untreated controls. Up on increase in time of UV irradiation, total flavonoid content increased in both the mushroom varieties. This study revealed a threefold increase in flavonoid content in *Pleurotus ostreatus*, rising from 1.2 mg of quercetin/g in the control to 4.1 mg of quercetin/g after UV exposure for 120 minutes. Conversely, a sevenfold increase in quercetin content was observed in Agaricus bisporus, surging from 0.45 g (control) to 3.2 g following 120 minutes of UV exposure. (**Table 2**). A study reported by Barros et al.[18] stated that the stages where the fruiting bodies represented immature spores revealed a higher content in phenol and flavonoid content. According to Geosel et al.[19], the low molecular weight antioxidants - flavonoids are able to prevent different types of health disorders in humans. Hence enhancements of flavonoids add nutraceutical value of the mushrooms. Tiwari et al.[20] have also reported the enhancement in vitamin D_2 content along with other nutraceutical constituents like antioxidants, total phenols, and flavonoids, in dried mushroom *Lentinula edodes* (*L. edodes*) powder using ultraviolet B (UVB) irradiation. This corroborates our findings of enhancement of functional food ingredients viz phenolics and flavonoids in mushrooms by UVB radiations for practical utility.

Table 2. Total Flavonoid content of UV irradiated mushrooms.

Time of UV irradiation (minutes)	Total flavonoid content in *Agaricus bisporus* (mg of Quercitin equivalents/g DW)	Total flavonoid content in *Pleurotus ostreatus* (mg of Quercitin equivalents/g DW)
0 (Control)	0.45 ± 0.93	1.2 ± 0.14
5	1.52 ± 2.58	2 ± 1.95
15	1.71 ± 1.03	2.3 ± 2.98
30	1.73 ± 1.02	2.8 ± 0.286
45	1.8 ± 2.14	3.5 ± 0.37
60	2.8 ± 2.5	3.9 ± 0.009
120	3.2 ± 1.68	4.1 ± 1.03

3.3. Enhancement of Folic acid of UV irradiated mushrooms

In general, the folic acid level was found higher in *Pleurotus ostreatus*(30.5 ± 0.107 ug/g DW) compared with *Agaricus bisporus* (20.05 ± 1.86 ug/g DW). With the increase in time of UV irradiation, folic acid content increased in both the mushroom varieties (**Table 3**). In *A bisporus* the folic acid levels increased to 30.8 ± 2.14 µg/mg DW whereas in *P ostreatus* it reached a level of 49.63 ± 3.6 µg/mg D. One plausible explanation for the enhancement of folic acid levels could be the cleavage of conjugate of folic acid upon UV exposure thereby enhancing the content in extracts of mushrooms. The folate composition of mushrooms has been studied by Phillip et al.[21]. To our knowledge, the effect of short-term UV exposure of mushrooms on their folic acid levels has not been reported. This finding is of relevance to administer mushroom based recipes to the population's deficient of vitamin B9. Bandi and Kola[22] have shown the enhancement of the antioxidant activity of mushrooms in irradiated mushrooms than in non-irradiated mushrooms. But they have not attributed it to folic acid in particular.

Table 3. Folic acid content of UV irradiated mushrooms.

Time of UV irradiation (minutes)	Folic acid content in*Agaricusbisporus*(µg/mg DW)	Folic acid content in *Pleurotusostreatus*(µg/mg DW)
0 (Control)	20.05 ± 1.86	30.5 ± 0.107
5	25.6 ± 0.56	35.2 ± 0.28
15	25.95 ± 2.48	37.45 ± 0.83
30	26.92 ± 0.109	40.25 ± 0.465
45	28.36 ± 0.106	45.6 ± 0.472
60	29.84 ± 1.42	47.45 ± 2.36
120	30.8 ± 2.14	49.63 ± 3.6

3.4. Enhancement of vitamin D upon UV irradiation of mushrooms

The impact of UV-C irradiation on the conversion of ergosterol to vitamin D2 in mushroom has been reported[23]. **Table 4** shows the absorbance of Vitamin D2 versus the time of irradiation. The exposure of UV light for 60 minutes resulted in the highest absorbance of vit D2 at 0.81 OD in *Pleorotus osteatus* compared to 0.46 in *Agaricus bisporus*. The absorbance of vitamin D2 under UV light increases gradually as the time of exposure increases up to 50 minutes and remains constant up to 60 minutes of irradiation (**Figure 1**). The high values of absorbance imply high concentration of vitamin D2[24]. The difference between the UV-C and UV-A absorbance values can be ascribed to a higher efficiency of vitamin D2 conversion at exposure to UV-C during the growth than UV-A light[25]. Similarly, Edward et al.[26] reported enhanced Vitamin D2 levels in Oyster Mushrooms, exposed to 245 nm and 365 nm UV-light during the growth period. Gallotti et al.[27] found that selecting mushroom species with high nutraceutical benefits from irradiation to enhance their potential properties to inhibit oxidative and glycation processes responsible for human diseases. Extrapolating this to our studies with the preliminary screening for superior germplasm to couple it with UV treatments will result in the enhancement of vit D2, folic acid, phenolics and flavonoids to increase the nutraceutical value.

Table 4. Absorbance values of Vitamin D^2 solution of mushroom samples differently exposed to UV-Light at 450 nm.

Time of UV irradiation (minutes)	Absorbance value of Vitamin D2 (*Agaricus bisporus*)	Absorbance Value of Vitamin D2 (*Pleurotus ostreatus*)
0 (Control)	0.21	0.29
10	0.23.	0.38
20	0.27	0.45
30	0.35	0.53
40	0.39	0.65
50	0.45	0.81
60	0.46	0.81

3.5. Quality assessment of edible polysaccharide-coated *Agaricus bisporus*

Assessment of the coating of mushrooms with edible polysaccharides was done by measuring the water loss and microbial quality analysis upon storage for 14 days (**Table 5**). It was noted that the weight loss was minimal in the mushrooms treated with ascorbic acid and coated with agar, as well as ascorbic acid treated and coated with gelatin. The other treatments were relatively ineffective. Interestingly the surface microflora analysed was also minimal in these treatments when compared to others. Spoilage was highest in CMC-coated mushrooms with 5.98 × 106 microbial count. When compared to the microbial cell count in control, the samples of mushrooms coated with Agar after the Ascorbic acid treatment, and those coated with Gelatin after the Ascorbic acid treatment, were better preserved as evident by low microbial count (**Table 6**). It may be concluded that the ascorbic acid pre-treatment followed by coating with agar or gelatine could be of value in extending the shelf life of mushrooms. Our study has implications for preserving this perishable commodity from deterioration during the passage through the supply chain. Esmaeili et al.[28] have evaluated modified atmospheric packaging containing 500 ppm cinnamon essential oil, aloe vera concentrated gel (1:3 with distilled water), calcium lactate, and ascorbic acid on the shelf life of strawberry. Nelson et al.[29] found the use of nanofilm, as well as the modification of the atmosphere, had a positive effect on the control of mushroom respiration rate and an improvement in its physical, chemical, and mechanical properties. Borges et al.[30] studied the Microbiological Assessment of White Button Mushrooms with an Edible Film Coating they reported that Coated mushrooms had less mass loss and color change, and had better texture after 14 days. Microbiological analysis revealed that the coating had no antimicrobial activity. Overall, the coating improved the shelf life of the coated mushrooms. Rondanelli et al.[31] reported that Coated mushrooms had less mass loss and color change, and had better texture up to 14 days. Overall, the coating improved the shelf life of the coated mushrooms but had less effect on the microbial community Pleșoianu et al.[32] who has also reported similar results of weight loss of mushrooms during storage has attributed to moisture evaporation and respiration.

Table 5. Weight loss in polysaccharides coated mushrooms after 14 days of storage.

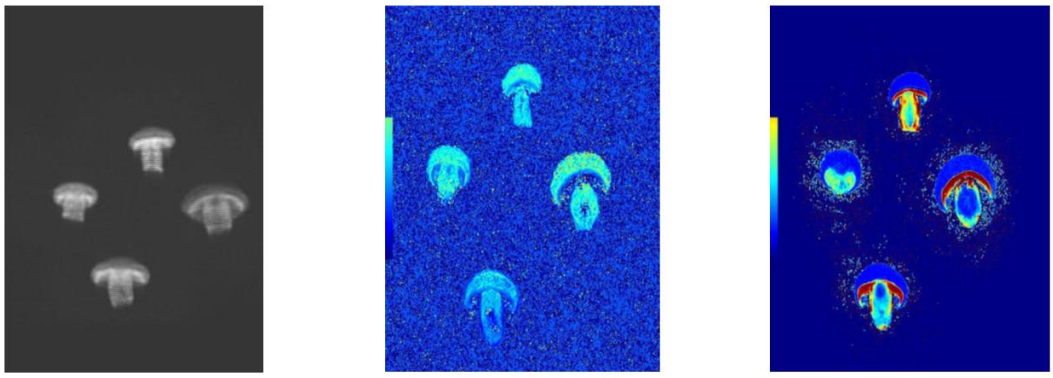
Coating	% Loss in weight
Control (Non-coated).	48
Ascorbic acid treated and non-coated.	48
Agar coated	50
Ascorbic acid treatment followed by Agar coating.	27
Gelatin coating	30
Ascorbic acid treatment followed by Gelatin coating.	25
CMC (Carboxy methyl cellulose) coating.	52
Ascorbic acid treatment followed by CMC coating.	46

Table 6. Microbial cell count in coated mushrooms after 14 days.

Coating	Microbial population (CFU/g/plate)
Control (Non-coated).	3.23×10^3
Ascorbic acid treated and non-coated.	2.6×10^2
Agar coated.	6.52×10^2
Ascorbic acid treatment followed by Agar coating.	1.32×10^2
Gelatine coating.	1.92×10^2
Ascorbic acid treatment followed by Gelatine coating.	1.22×10^2
CMC (Carboxyl methyl cellulose) coating.	5.98×10^4
Ascorbic acid treatment followed by CMC coating.	4.22×10^4

3.6. MRI scanning of mushrooms

For the first time, we have demonstrated that mushrooms can be imaged by MRI to profile the lipids and water in the sample. The experiments were carried out on a Siemens 1.5T Magnetom Avanto MRI scanner. Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) was performed to spatially map lipid content. This technique has been routinely used in medical diagnosis for lesions and sclerosis detection . MPRAGE was performed [Slice thickness = 1.85 mm, TR/TE = 1650/2.9 ms, Averages = 1, Field of View = 128 mm]. T_1 maps were obtained to estimate lipid content with the following parameters [Slice thickness = 5 mm, orientation = axial, TR/TE = 20/4.4500 ms, Averages =1, Flip Angles = 9.47. Field of View = 128 mm]. T_2 weighted imaging was performed to estimate moisture content with the following parameters [Slice thickness = 5 mm, orientation = axial, TR/TE = 2600/22 ms, Averages = 1, Flip Angle = 180, Field of View = 128 mm]. A typical image of MRI has been shown for the lipids in the sample (Fig 1). Region of interest (ROI) values obtained for various treatments of coated mushrooms at the end of 14 days were converted to the percentage of lipids. At the end of the storage period of 14 days, the values of lipids obtained through MRI as ROI were converted to 100 % in uncoated controls, and the relative values in the treatments are tabulated and presented in **Table 7**. This result showed that mushrooms coated with agar with prior treatment with ascorbic acid have higher lipids, as the keeping quality of this set of treatments is the best among the treatments tried. Spoiled mushrooms have lower lipids due to the deterioration of cells in mushrooms.



(a) (b) (c)

Figure 1. MRI Scan of mushrooms coated with Gelatin. **(a)** T_1 MPRAGE; **(b)** T_1 MAP; **(c)** T_2 MAP. T_1 MAP gives lipid profile and T_2 MAP gives water profile of mushrooms.

Table 7. Region of interest values of the T1 MAP obtained for lipid profile in the coated mushrooms after 14 days of storage.

Treatment	Lipid values %
Control not coated	100
Agar coated	115.3
Ascorbic acid treatment followed by Agar coating	120.77
Gelatin coated	105.2
Ascorbic acid treatment followed by Gelatin coating	111.01
CMC coating	98.5
Ascorbic acid treatment followed by CMC coating	98.3

4. Conclusion

The enhancement of the nutraceutical value of mushrooms upon UV treatment was also evident from the enhancement of phenolics, flavonoids, folic acid, and Vit D_2 levels. This has implications in utilizing the UV-treated mushrooms for the vulnerable populations deficient in nutraceuticals mainly Vit D_2 and Folic acid. The result from the present study has shown value addition to the shelf life of mushrooms upon treatment with antioxidant-ascorbic acid followed by coating with agar or Gelatin. The shelf life of such coated mushrooms could be 14 days compared to normal deterioration time of 4–5 days. The use of MRI technique to assess the quality of mushrooms has opened up a quick screening method adoptable in an automated manner. This study provides a sustainable strategy to improve the nutraceutical potential of dried edible mushroom powder, in terms of antioxidant activities, bioactive constituents, etc., particularly vitamin D_2, which holds a significant place in the diet. The studies reported here concerning the enhancement of the nutraceutical profile and extension of keeping quality have practical utility from the point of view of large-scale production in the mushroom industry.

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Conflict of interest

The authors declare no conflict of interest.

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