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ASSESSMENT OF BUTTON AND OYSTER MUSHROOM NUTRITIONAL QUALITY USING VARIOUS DRYING METHODS

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ABSTRACT

Edible mushrooms are in high demand due to their flavor and nutritional benefits. Mushrooms are a rich source of carbohydrates, with high fibre content and modest protein content containing the majority of the amino acids, and vitamins. This study was conducted to evaluate the effects of different pretreatment drying methods on the nutritional quality of dried mushrooms (Button and Oyster mushroom) and also to estimate the Vitamin D_2 content. The experiment was carried out based on the three pretreatment techniques: SD, HD and UV + HD. Significant differences in proximate composition were observed between the fresh and dried mushroom samples. The average mean value of crude protein, crude fat, crude fiber, ash, and carbohydrates of dried mushroom samples were 33.9, 19, 5.7, 10.8 and 98.1% respectively and found to be statistically significant too. Sundried oyster mushrooms had their vitamin D_2 level increased by two folds in comparison with sundried button mushrooms. Finally, the intake of these mushrooms should be encouraged as a complement to impoverished people's main foods. As a result, addressing nutritional issues in children, pregnant women, and immune-compromised individuals is a necessity.

1. Introduction

Vitamin D, often known as the "Sun Vitamin," is a fat-soluble vitamin that aids in bone metabolism and has immune-modulating effects. Ergocalciferol (D_2) and Cholecalciferol (D_3) are the two major forms of Vitamin D required for humans. The current Adequate Intake (AI) for Vitamin D for most adults is 800 IU/day whereas for infants is 600 IU/day (Phillips et al, 2013). Vitamin D is found naturally in a few foods, and it may also be synthesized endogenously when UV rays from the sun impact the skin and cause vitamin D production. Vitamin D insufficiency, on the other hand, is the most under diagnosed and undertreated nutritional condition around the world. Especially in tropical countries like India, Vitamin D deficiency prevails in epidemic proportions of 70% to 100% in the general population (Sanwalka et al, 2016). Vitamin D deficiency is believed to have a predominant role in India's high rates of infections like arthritis, rickets, osteoporosis, cardiovascular disease, diabetes, cancer, and TB (Tuberculosis). (Ritu et al, 2014).In order to overcome Vitamin D deficiency in humans, Vitamin D has to be supplemented in diet. Vegetable sources like yeast, mushroom, cheese are rich in Precursor of Vitamin D_2 whereas animal sources such as fish, egg, beef liver, seafoods etc were precursors of vitamin D_3 (O'Mahony et al, 2011). Ergosterols, a precursor of vitamin D_2, are found in mushrooms and other plant foods. The lack of natural plant-based vitamin D_3 sources has

necessitated the development of alternatives to increase vitamin D_3 consumption. Vitamin D_3 is added to most meals, while vitamin D_2 (ergocalciferol) is commonly found in nutritional supplements and vegetarian goods such as soy milk (Phillips et al, 2013). Hence, supplementation of Vitamin D_2 in foods gains attention.

Commercially, Vitamin D_2 is made by exposing yeast to ultraviolet light (UV), which transforms the fungal sterol ergosterol to ergocalciferol. Ergosterol is found in all mushrooms, although the amount of vitamin D_2 varies greatly. Among them, oyster mushrooms reported to have higher Ergosterol content than others (Philips et al, 2011). Higher Ergosterol content and larger surface area makes mushroom more preferable for Vitamin D fortification. The oldest method used for food preservation is the drying method. The drying process reduces the moisture content of food, which inhibits the microbial development. The drying process is a good way to keep solid meals safe for a long period on a large scale (Muhamad et al, 2019). Pre-treatments are used to speed up the drying process, improve food quality, and increase food safety (Longvah et al, 1998). This drying operation is carried out for the following reasons: to extend the shelf life, to improve the quality and to reduce the volume which results in reduced packaging and transportation cost (Vijayan et al, 2017).

The objective of this work is to investigate the effect of direct sunlight, UV treatment and Hot air UV treatment on the macronutrient contents of *Pleurotus ostreatus* and *Agaricus bisporus* and also to examine the level of Vitamin D_2 content.

2. Materials and methods

2.1. Materials

2.1.1. Collection of Fresh Mushroom

All mushrooms were selected according to the size of the cap (d = 50 mm) and it was cleaned thoroughly to remove adhering matter. After washing with tap water, it is dried so that the moisture absorbed by the mushrooms does not influence the analysis (Balan, V et al., 2021). Then, the samples were stored at 4 °C in a refrigerator and processed within 24 h after harvest.

2.2. Methods

2.2.1. Pre-treatment Processes

Pre-treatment is required for foods to be treated before the canning process; pre-treatment varies per food. The pre-treatment process aids in the preservation of color, nutrition, taste, and overall quality.

2.2.2. Drying Process

The whole mushroom was washed thoroughly under running tap water to eliminate any adherent extraneous debris. The brown and damaged portions of the mushroom were removed. Washed and cleaned mushrooms were taken for the experimental studies. The mushroom was dried in three various ways: sun-drying (SD), Hot air oven drying (HD) and Ultraviolet radiation + Hot air oven drying (UV + HD).

2.2.3. Sun Drying (SD)

Around 200 g of button and oyster mushrooms were taken for sun drying after washing. Then the button and oyster mushrooms were chopped into small pieces. After chopping, the mushrooms were placed in separate plastic trays and dried in the open sun rays from morning to evening at an ambient temperature of 32±5 ℃ for 2 days. The mushrooms were ground dried in a mixer and made as a fine powder. Then, the finely powdered mushroom was stored in a separate plastic container at room temperature (Maray et al., 2018).

2.2.4. Hot Air Oven Drying (HD)

Hot air oven drying was done by taking 200 g of a button and oyster mushrooms were chopped into small pieces. After chopping, it was placed in petri dishes and kept in the oven. The temperature was kept constant at 62 ℃ respectively for 2 days. Once the mushroom turned into a brown color due to the removal of moisture content, it was made as a fine powder and used for analysis (Maray et al., 2018).

2.2.5. Ultraviolet + Hot Air Oven Drying (UV+HD)

The final pre-treatment process was performed using ultraviolet radiation followed by hot air oven exposure of chopped mushrooms. Initially, the laminar hood was sterilized using ethanol, and then chopped mushroom was made to treat with UV rays for 5 mins with intensity 0.6–1 W/m^2. Being treated with UV rays, button and oyster mushroom was reduced in a hot air oven by keeping the temperature at 62 ℃ for 2 days. Then the dried mushroom was grinded and stored at room temperature (Francesca Gallotti et al., 2020).

2.2.6. Determination of Carbohydrate

The mushroom sample of about 100mg was taken and it was hydrolyzed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl. From the hydrolyzed sample, 0.5 ml was taken for test analysis. Consequently, the standard solution was prepared by making the different concentrations as a working standard. Each test tube was made up to the volume of 1ml in all test tubes by adding distilled water. Then, 4 ml of anthrone reagent was added. Thereafter, it was heated for about 8 mins in a boiling water bath till a bluish green color appears. Then cooled rapidly and the absorbance was taken at 630nm (Sadasivam 1996).

2.2.7. Determination of Crude Protein

Different dilutions of BSA solutions were prepared by mixing protein standard solution and water in a test tube. The unknown samples were taken in another test tube. All the test tubes were made up to the volume of 3 ml by using distilled water. The alkaline copper reagent of 4.5 ml was added to each test tube and incubated for 10 mins at room temperature. After that, 0.5 ml of folin – ciocalteu reagent was added to all test tubes and heated till the formation of bluish - green color. Then, the absorbance was measured at 630 nm (Nielson 2017).

2.2.8. Determination of Lipid content

The sample (80 g) was taken in a round bottomed flask; it was extracted using 200 ml of acetone of boiling point (56 °C). The extraction process continues for about 2 hours till most of the solvents are distilled from the flask into the extractor. The amount of lipid was calculated from the residue remaining after evaporation of solvents (Min et al., 2010).

$$\%\text{Acetone extract} = \frac{(\text{Weight of flask} + \text{Weight of extract}) - (\text{Weight. of flask}) \times 100.}{\text{Weight of sample}} \quad (1)$$

2.2.9. Determination of Ash

The sample (2 g) was ignited at 400 °C for 4 hours or until whitish-grey ash was formed in a muffle furnace. The crucible was then weighed after being inserted in the desiccator (Ismail et al., 2017).

$$\%\text{Ash} = \frac{(\text{Weight of crucible} + \text{ash}) - (\text{Weight of crucible})}{\text{Weight of sample}} \quad (2)$$

2.2.10. Determination of Moisture

The sample (2 g) was taken in a petridish was dried in an oven to remove moisture content for 60 ℃ for about 36 hours. Then, it was cooled using a desiccator and weighed. The drying and weighing process continues until a constant value is achieved (Nielsen 2010).

$$\%\ \text{Moisture} = \frac{(\text{Weight of sample} + \text{dish before drying}) - (\text{Weight of sample} + \text{dish after drying}) \times 100}{\text{Weight of sample taken}} \quad (3)$$

2.2.11. Determination of Fiber

The sample (5 g) was taken in a beaker. In which, 50 ml of sulphuric acid (H_2SO_4) and potassium hydroxide (KOH) solution was added and boiled for 30 mins. After that, the samples were washed using distilled water after that sieve. Then, 20ml acetone was added and it was left undisturbed for 20 mins. And then, the samples were filtered and dried for 30 mins in a hot air oven (Gul et al., 2009).

$$\%\ \text{Fiber} = \frac{(\text{Weight of crucible with dry residue}) - (\text{Weight of crucible with ash}) \times 100}{\text{Weight of sample taken}} \quad (4)$$

2.2.12. Estimation of Energy value

Food's energy content is an important factor to consider. The calorie content per weight of food is referred to as the energy density of the food. Carbohydrates are the most essential of the three primary nutrients needed for energy. The body can use protein and fats for energy when carbohydrate has been depleted. Our body breaks down nutrients into smaller components and absorbs them to use as energy. The energy available in a mushroom powder was calculated by multiplying the number of grams of carbohydrate and protein was multiplied by 4 and fat was multiplied by 9 respectively. Then add the results together (Schakel et al., 1997).

Energy (in Kcal) = 4× (Carbohydrates and Proteins in grams) + 9 × (Fat in grams)

2.2.13. Quantification of Vitamin D_2

The Vitamin D_2 content was analyzed by high performance liquid chromatography (HPLC) using the methodology previously reported by (Philips et al, 2011). Briefly, mushroom samples with [2 h]-vitamin D_2 added as an internal standard which has been saponified in methanolic KOH and it was purified by solid-phase extraction. HPLC was performed to isolate the vitamin D fraction, and vitamin D_2 was then separated by reverse-phase HPLC with UV detection at 265 nm and quantified based on the ratio of sample peak area to [2 H]-vitamin D_2 ratio relative to an external standard curve from analysis of vitamin D_2 standards spiked with an equivalent amount of internal standard.

2.2.14. Statistical Analysis

The statistical analysis was done using one-way ANOVA. The P value and F value were calculated to measure the significance of the results obtained.

3.Results and discussions

3.1. Effect of Pre-treatments methods for Mushroom

Three different drying methods were performed in this study: Sun drying at ambient temperature 32.5±5 °C, Hot air oven drying at 62 °C and finally UV+ HD drying at 62 °C were carried out on button and oyster mushroom (Figure 1 to 6).

Figure 1. Pre-treatment of Button mushroom (BM) by Sundrying

Figure 2. Pre-treatment of Oyster mushroom (OM) by Sundrying

Figure 3. Hot air oven drying (HD) treatment for Button mushroom (BM)

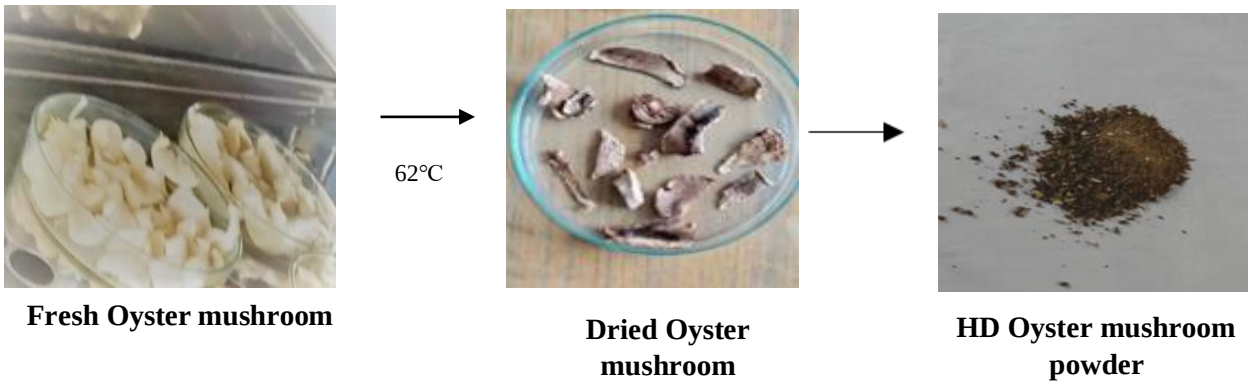

Figure 4. Hot air oven drying (HD) treatment for Oyster mushroom (OM)

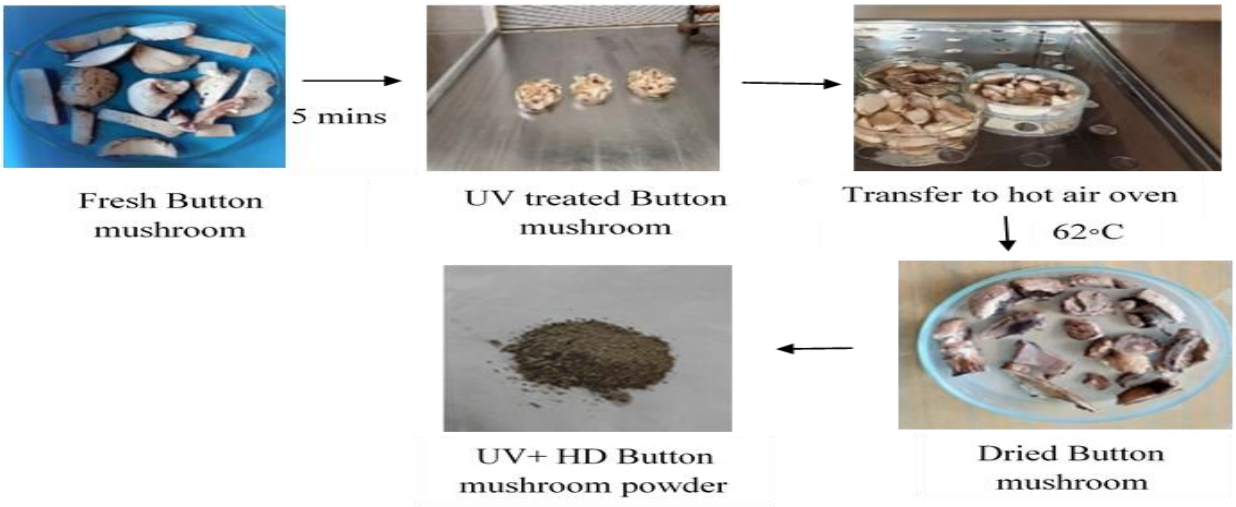

Figure 5. Pre-treated Button mushroom (BM) under UV followed by Hot air oven drying (HD) treatment

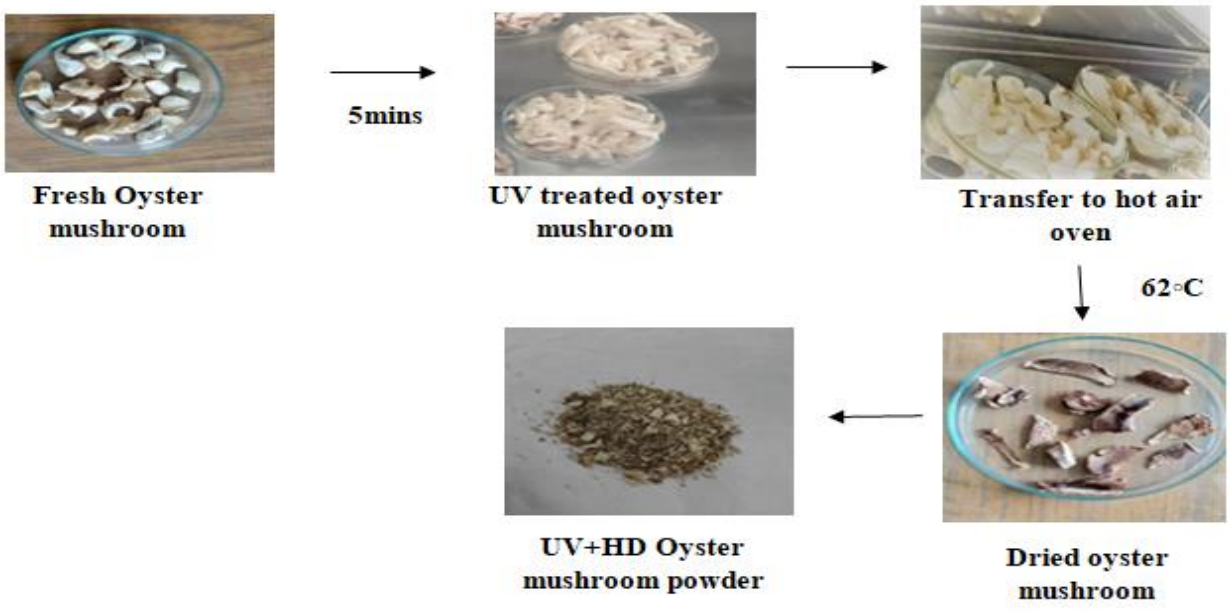

Figure 6. Pre-treated Oyster mushroom (OM) under UV followed by Hot air oven (HD) treatment

3.2. Proximate composition of dried button and oyster mushroom

Proximate analysis was carried out on two edible mushroom species: *Pleurotus ostreatus* and *Agaricus bisporus* for both pretreated and fresh mushrooms. Proximate analysis of the mushrooms including moisture, fat, fiber, ash, protein and carbohydrate were determined. The fresh mushroom proximate analysis values are represented in Table 1.

Table 1. Composition of fresh mushroom

Analysis	Fresh BM*	Fresh OM*
Carbohydrate	46.17	48.16
Protein	33.48	28.85
Fat	3.36	2.47
Ash	5.7	9.76
Fiber	20.9	12.87
Moisture	92.45	88.75

*BM – Button mushroom, OM – Oyster Mushroom

3.2.1. Carbohydrate content

Carbohydrate plays a significant role in food since it provides energy for the human being. Polysaccharide is the major carbohydrate present in mushrooms and also it's determined to have immunomodulation and antitumor properties (Chang et al, 1982). The present study revealed that *Agaricus bisporus* (98.1g) determined to have significantly higher carbohydrate content compared to *Pleurotus ostreatus* (89.9g) under sun drying treatment whereas the lowest value (16.2g) was recorded for the samples dried in HD button mushroom (16.2g) and UV + HD oyster mushroom (15.4g). The values were represented in figure 7. The significant amount of carbohydrate is found in the dried mushroom which is in correlation with the values reported by (Chandravadana et al, 2005).Hence the sun dried mushroom serves as good dietary fiber with calorific value. Because of the concentration of nutrients, the carbohydrate percentage increases as the moisture content decreases during drying. The increase in protein in dried samples is caused by the dehydration of the water that exists between proteins. Carbohydrates, like the majority of heat-sensitive nutrients in food, tend to lose their function. This explains why the total carbohydrate content of vacuum and oven-dried samples was lower than that of free samples (Sim et al., 2017).

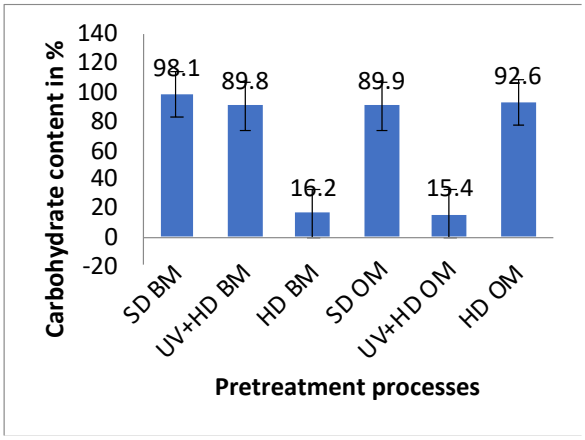

Figure 7. Analysis of Carbohydrate content in dried mushroom

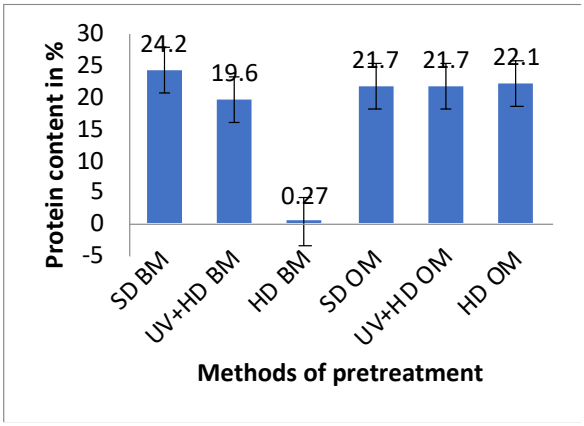

Figure 8. Analysis of protein content in dried mushroom

3.2.2. Protein Content

Protein accounts for more than half of the total nitrogen in mushrooms, and its quantity varies depending on the species, substrate composition, and size of pileus and the harvest season. Mushrooms have 19 to 35 percent protein by dry weight, compared to 7.2 percent for rice, 13.2 percent for wheat, and 25.2 percent for milk. In this study, the protein content ranged from 19.6 g to 33.9 g. Protein content varies widely across mushroom species, ranging from 11 to 42 g/100 g dry fruit bodies.

Except for hot air oven drying of the button mushroom, other pretreated processes identified to show similar protein content (Figure 8). According to a prior study, the water solubility index rises as particle size decreases, owing to greater surface area and enhanced protein solubilization. This means that superfine mushroom powder has a higher concentration of nutrients soluble in water than coarser powders, making it easier to utilize as a food ingredient in recipes (Wu et al., 2012). Mushrooms have a high protein content that rivals that of animal protein sources. The protein content of mushroom mycelia was higher than that of the available fruiting bodies. Nonetheless, the protein content of mushrooms can be influenced by a variety of factors such as their developmental stage, mushroom type, cultivation location, and post-harvest treatments (Sim et al., 2017).

3.2.3. Fat content

Generally, mushrooms tend to have lower fat content but they are found to have polyunsaturated fatty acids which are essential for human health and also it contributes to the reduction of serum cholesterol level. Especially, the higher content of linoleic acid is one of the reasons for considering mushrooms as a healthy food (Goyal *et al*, 2015). In general, the fat content of cultivated mushrooms ranges between 0.6 – 3.1% as dry weight and also the fat content widely varies depending on the mushroom type and cultivation method. The crude fat contained in this study ranged from 1.1g to 1.9g. The fat content was higher in mushrooms treated with SD BM, UV+HD BM and SD OM with values of 19 g, 19 g and 17 g respectively (Figure 9 and 10). Lower fat content was observed in UV+ HD OM (11.9 g). The estimated fat content values in this study were reported to be the same by (Singh et al, 2016). While comparing the *Agaricus bisporus* and *Pleurotus ostreatus*, the lower fat contents were found in various pretreatments of *Pleurotus ostreatus*. It emphasizes the role of substrate on the fat content used for the cultivation of oyster mushrooms (McGrath et al, 1982).

3.2.4. Fiber Content

Edible mushrooms showed a hypocholesterolemic impact in the diet, possibly due to dietary fibers such as beta-glucans. This may promote intestinal motility

while lowering bile and adsorption of cholesterol levels. The percentage ranges from 4% to 13% of total dietary fiber consumption. The sum of the nondigestible carbohydrates (chitin) and lignin (plants) makes up the total dietary fiber (TDF). Usually, the chitin content of the mushroom was greater in *A. bisporus* fungus that causes *Pleurotus* (Manzi et al, 2004). While comparing dried mushrooms to fresh or frozen mushrooms, fresh mushrooms have higher beta-glucan content than dried ones. It denotes that the heat treatment affects the beta-glucan content. Afiukwa et al. found that *Plerotus ostreatus* had a fiber content of 29.00%, which is much greater than the values reported in this investigation. Sun dried *Pleurotus spp* has 12.59 percent crude fibre compared to 12.58 percent for oven dried *Pleurotus spp* mushrooms, according to (Dunkwal et al, 2007).

3.2.5. Ash content

Ash is defined as fully non-burnable inorganic salts. Potassium and phosphorus are the principal ash elements in mushrooms. According to studies, mushrooms are high in minerals and can also be a superior source of minerals than vegetables. Ash content data of the dried mushrooms are represented in (Figure 9, 10). HD and UV + HD dried button mushroom samples show the ash content of 10.2 g and 8.6 g respectively and also the content is statistically lower than that of SD BM (10.8 g). The study by (Muyanja et al, 2014), sun-dried and oven-dried treated powders had ash levels ranging from 0.44 to 0.54 g/g dry matter. Pretreatment oyster mushrooms showed the ash content value of 10.7g and 10.2 g in SD and HD dried samples which is statistically higher than the contents of UV + HD dried samples (4.6 g). These findings exactly matched with (Singh et al, 2016) study. The sun dried pretreatment is more efficient for both button and oyster mushroom as per ash contents.

3.2.6. Moisture content

The elimination of moisture content during processing may boost nutrient concentration in the mushroom by extending its shelf life. The weight of the mushroom fell fast as the temperature increased; the drying temperature had a significant impact on the moisture removal of the mushroom. The moisture content was almost remaining the same for the mushroom treated in all conditions in this study. The current study's findings revealed that the moisture content is low for both oyster and button mushroom (Figure 9 and 10) as compared to (Sunday et al, 2016 and Tolera et al, 2017). While using drying methods, case hardening can occur, resulting in greater moisture content in samples than when utilizing traditional drying methods under identical treatment circumstances.

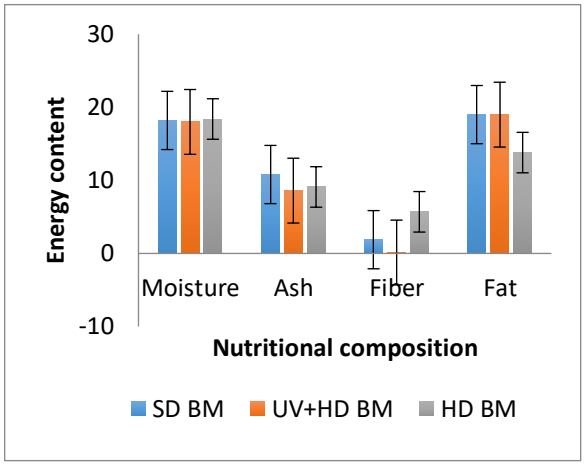

Figure 9. Graphical representation of proximate analysis of dried button mushroom

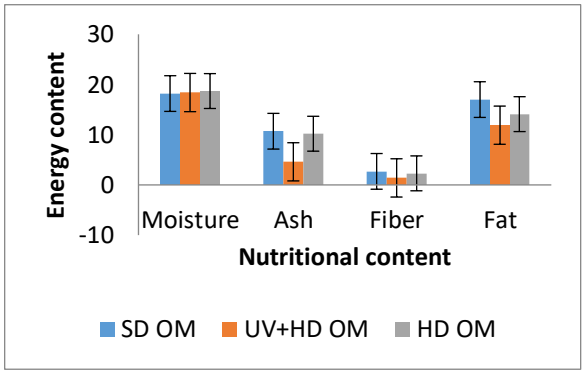

Figure 10. Graphical representation of proximate analysis of dried oyster mushroom

3.2.7. Estimation of energy content in dried mushroom

A characteristic of food is energy density or the amount of energy (in kilocalories) per unit of food (in gram). As a result, it may be determined by dividing total kilocalories by total gram for meals, and the entire diet. Various dietary components, such as macronutrient and water content, have an impact on energy content. Water has the most impact on a food's energy content since it adds significant weight without providing energy. Fat is the most important macronutrient due to its high energy level when compared to protein or carbohydrate. The sundried mushrooms determined to have significant energy content compared to other pretreatment methods in both the mushroom varieties in this study (Figure 11). The higher energy content in sundried mushroom is due to the presence of higher protein content (Refer figure 8).

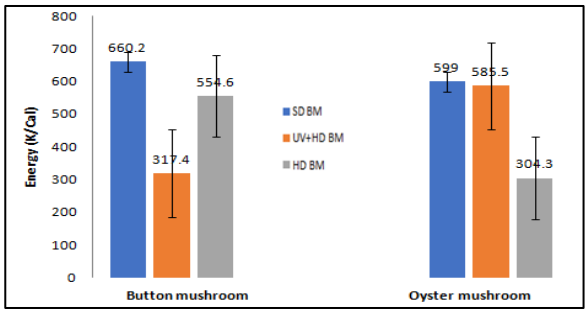

Figure 11. Overall energy calculation for drying processes

3.2.8. Quantification of Vitamin- D_2 content

The Vitamin D_2 content was assessed for sun-treated button and oyster mushroom, since the proximate composition values found to be appropriate for sun dried mushroom rather than other drying methods.

The vitamin D_2 concentration was found to be 83.3 µg/100 g and 45 µg/100 g for sun dried oyster and button mushroom in the present study. The quantity of ergosterol present in dry matter of sun dried mushroom was summarized in Table 3. The study by (Nolle et al, 2017) showed that sun-dried mushrooms contained 36 µg/g dry matter vitamin D_2; whereas the (Urbain et al, 2015) study experimentally proved that the vitamin D_2 content increased to 17.6 µg per 100 g of fresh mushroom.

Table 2. Quantity of ergosterol present in sun dried mushroom

Sample	**Sundried Button mushroom**	**Sundried Oyster mushroom**
Ergosterol(µg/100g)	45	83.3
SD	34.87312	31.67832
RSD(%)	1.2153	1.1864

The reason behind the result discrepancy can be correlated with the mushroom variety, ergosterol content, exposure dose and time as well as temperature. The quantity and quality of solar energy reaching the earth's surface is affected by latitude and season, which has an impact on vitamin D synthesis (Webb et al, 1988). Sun drying is a realistic approach for the natural creation of ergocalciferol in mushrooms and a great vitamin D source for vegans, even if commercial UVB radiation boosts vitamin D_2 synthesis in mushrooms.

3.3. Statistical analysis

The statistical analysis was performed for the proximate analysis values using single factor ANOVA. The values were found to be statistically significant with $p < 0.05$.

Table 3: Statistical analysis performed for the proximate values

Source variation	**SS**	**df**	**MS**	**F value**	**P-value**	**Fcrit value**
Between groups	333.21	1	333.2	7.97	0.02	5.32
Within groups	334.35	8	41.79	-	-	-
Total	667.57	9	-	-	-	-

4. Conclusions

The nutritional quality of the dried mushroom was statistically significant when varied pretreatments and drying techniques were used. Among them, the sun drying method was effective in maintaining and increasing the amount of vitamin D_2 in the dried mushroom. The sun dried mushrooms represent a prominent source in providing dietary vitamin D_2. This is especially important because just a few foods provide naturally high levels of vitamin D_2 content, such as salmon (12.4 mg/g), herring (15.4 mg/g), and egg yolk (7.8 mg/g). As a result, a little quantity of sun dried mushrooms can provide the necessary daily consumption of vitamin D_2 (600-800 IU) for humans. Drying mushrooms in low-cost, locally available sun drying methods presents a viable alternative to UV treatment method for natural vitamin- D_2 enrichment, which might lead to the production of safe vitamin D_2 enriched foods. As a possible future work, the other edible mushroom species must be evaluated in order to optimize processing and preservation methods for the distribution of high-quality dried mushrooms in order to promote mushroom production, preservation, and consumption in developing nations.

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