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Vitamin D_2 retention and toxicity in dried oyster mushroom (*Pleurotus ostreatus*) powder

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Mushrooms are a rich source of ergosterol (precursor of ergocalciferol) which can be converted to vitamin D_2 in presence of natural or artificial ultraviolet light. Mushrooms are the only non-animal based food of vitamin D. The main objectives of the study were to optimize vitamin D_2 retention from oyster mushrooms and to analyse its toxic effects. Oyster mushrooms were cleaned, sliced 1x1 cm^2 and divided into four portions for subjecting to different methods of drying namely sundried, cabinet dried, freeze dried and 30 minutes exposed to sunlight and freeze dried. Vitamin D_2 was extracted from the dried powdered samples using ethanol as solvent extract by Ultrasonic Assisted Extraction. Vitamin D_2 retention was determined by HPLC using standard vitamin D_2. Crude extract of powdered mushroom was analysed for Brine shrimp lethality assay to analyse toxic effect using Potassium dichromate ($K_2Cr_2O_7$). This experiment revealed that the vitamin D_2 content was highest in sun dried oyster mushroom extract when compared to other drying methods, can be stored for a longer period of time and is also convenient for household purposes. Oyster mushrooms were found to be less toxic than $K_2Cr_2O_7$ since the LC_{50} value was >1 mg/ml. Hence, sundried oyster mushroom powder can be incorporated to food items or to enrich nutrient contents of foods which aids to overcome vitamin Ddeficiency.

Keywords : Ergocalciferol, *Pleurotus ostreatus*, retention time, toxicity, ultrasonic assisted ectraction

INTRODUCTION

Oyster mushroom is an edible fungus which is highly perishable in nature containing 80%-90% of moisture (Schill *et al.* 2021). They are grown indoors in mushroom houses, commonly on wheat/paddy straw and they can be cultivated throughout the year. White oyster mushrooms and grey oyster mushrooms are suitably grown in Tamil Nadu (TNAU Agritech Portal). It contains highly quality of protein, low calorie and also rich in B vitamins, vitamin C and other minerals which are necessary in our body (Nongthombam *et al.* 2021).

Although vitamin D_2 present naturally in fresh mushroom is very low or non-detectable amount, but rich source of ergosterol (precursor of ergocalciferol) which can convert to vitamin D_2 in presence of natural or artificial ultraviolet light in optimal range (Hu *et al.* 2020).

Drying is one of the easiest and cost effective methods of preservation to increase shelf life of mushrooms up to more than one year in airtight containers. There are different techniques of drying including sun drying, convection hot air drying, freeze drying, combination of quick microwave heating are adopted for drying mushrooms. Out of these methods, Sun drying is the traditional way of drying. However, the dried product may be affected while adopting sun drying due to environmental factors, insects, pests, weather, etc. (Van Hung *et al*. 2020). But people are still practicing sun drying method due to zero expenditure and convenient for day to day life. Sun drying not only reduced moisture content of the mushrooms but also an excellent source of vitamin D_2 **(**Nölle *et al.* 2017**).**

Vitamin D is a major fat soluble vitamin which is responsible for many functions in our body. Insufficient intake and less bioavailability of Vitamin D leading to deficiency and causes numerous health issues in worldwide (Kalpana *et al.* 2023). Vitamin D improves to synthesize

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calcium transport proteins in small intestines which elevating the absorption of calcium and thus decrease risk of rickets in young children and osteomalacia in adults (Cardwell *et al.* 2018). Sunlight is the major source of vitamin D but due to less exposure to sunlight which caused lower absorption of calcium in body (Narang and Sharma 2020). Additionally, Lectins contain in the edible mushroom has a potent of antiproliferative effect which has a new potential role in cancer treatments. (Perduca *et al.* 2020).

The main aim of the study is to optimize the retention of vitamin D_2 retention from various drying methods of oyster mushrooms and to analyse its toxic effect.

MATERIALS AND METHODS

Sample selection

Fresh oyster mushrooms were purchased from a local farm, Coimbatore, Tamil Nadu, India. Mushroom was harvested between April to May (summer mushrooms) dried in a sunny day

of month May of the year 2023 since UV index was extreme (11±1) during May in Coimbatore.

Sample preparation

Oyster mushroom was cleaned thoroughly and stalk was removed. Mushrooms were sliced into 1x1 cm^2 pieces and divided into four different samples based on drying methods.

A)Sun drying : Sundried the sliced mushrooms and gills up towards sunlight for 2 days from 10 am to 3 pm on sunny days in Coimbatore, the temperature in the range of 30°C-35 and ultraviolet index was extreme at 11±1.

B)Cabinet drying : Sliced mushrooms were dried in cabinet dryer for 8 hrs at a temperature 60±5.

C)Freeze drying: Sliced mushrooms were kept in lyophilizer for 2 days at - 53and vacuum pressure at 0.062 mbar. Lyophilization process retained colour, texture and appearance of the sample (Argyropoulos*et al*.2011).

D)Sun drying + freeze drying : Sliced mushrooms were sundried for 30 minutes (same condition as group A) and continued drying in lyophilizer (same condition as group C). The samples were termed as Sundried oyster mushroom (SDOM), Cabinet dried oyster mushroom (CDOM), Freeze dried oyster mushroom (FDOM) and Sun dried and Freeze dried oyster mushroom (SDFD) respectively.

The dried mushroom was finely powdered and screened through <0.5 mm mesh sieve and stored in an airtight container at < -4 for analysis.

Extraction of vitamin $D_2$from oyster mushroom powder by Ultrasonication-assisted extraction (UAE) method

The process of vitamin D_2 (*ergocalciferol*) extraction was performed by the protocol of Patil *et al.* (2018) with minimum modification at solvent usage, extraction time and speed.

One gram of powdered oyster mushroom from each drying method was taken separately in 100 ml glass beaker and 20 ml of ethanol was added to it (1:20 portion). Different oyster mushroom powder samples were ultrasonicated (SONICA® ULTRASONIC CLEANSER) for 30 mins at 60. After ultrasonication, the extracts were centrifuged (ThermoSorvall Legend XTR Refrigerated Centrifuge) at 8000 rpm for 15 minutes at 25. Separated the supernatant and dried in stirred water bath at the temperature of 60. The crude extract was diluted by adding 5 ml of ethanol solvent. 5 ml of each extracts were filtered with 0.4µm syringe filter and quantified the concentration of ergocalciferol by HPLC-UV.

Optimization of Vitamin D_2 by HPLC

The analysis of vitamin D_2 was performed on HPLC (Shimadzu prominence iLC – 2030c 3D plus). The mobile phase consisted of 95% acetronitrile and 5% methanol, it was pumped at theflow rate of 2 ml/minute (Patil *et al.* 2018). The extracted samples were filtered through 0.4µm syringe filter and analysed by HPLC-UV. The run time per same was 30 minutes at the wavelength of 265 nm and temperature of 35°C, retention time and peak were recorded. Standard ergocalciferol

(vitamin D_2) (Sigma Brand) - 10µl, 20µL,30µl, 40µl & 50µl was used to plot standard calibration curve. Five-points calibration curve was obtained using linear regression fit range from 10µl/mL to 50µl/mL (Y= 7348.7x - 2618.6) and R^2 value of standard vitamin D_2 was 1. HPLC was performed in PSG-BIRAC BioNEST Bioincubation Centre.

Vitamin D_2 content in different samples was quantified from the peak area and concentration by using the following formula given by Staffas and Nyman (2003):

$$\text{Cs µg/100 g} = \frac{\text{ADs x } mD_2 \text{ x 100}}{AD_2 \text{ x ms x F}}$$

Where,
Cs –Vitamin D content in the sample, ADs –Peak area of standard , mD_2 – Weight of Vitamin D_2 added in test portion (µg)
Peak area Rf= Concentration
ms – Weight of test portion (g)
F –Response factor (Rf)

$$\left\{ Rf = \frac{\text{Peak area}}{\text{Concentration}} \right\}$$

Toxic analysis by Brine Shrimps Lethality Assay

Sample preparation

The extraction process was adopted according to Tibuhwa (2017), where 0.5 g of freeze dried mushroom powder was added in a beaker followed by addition of 15 ml of ethanol, and was incubated in shaker incubator for 48 hrs at 50°C. The solution was filtered through cheese cloth, rinsed the beaker with 5 ml of ethanol and filtered again. Kept the filtered solution in water bath to vaporize at 70°C for 1 hr to get dry crude extract.

% Yield extracts was calculated based on dry weight as:

$$\% \text{ Yield} = \frac{\text{Weight of extract after ethanol evaporation } (W_1) \text{ x 100}}{\text{Weight of the extracted oyster mushroom } (W_2)}$$

Brine Shrimps Lethality Assay

One mg of crude extract was dissolved in 1 ml of distilled water (1mg/ml) to make stock solution. The stock solution of different volume of 100 µl, 250 µl, 500 µl, 1000 µl and 1500 µl were added to each beaker containing 25 ml saline solution respectively. 30 shrimps were introduced into each beaker containing different concentrations. The mobility of shrimps was observed at intervals of 1, 2, 4, 6 & 24 hours. For blank solution, 30 shrimps were added in Brine solution and Potassium dichromate was used for positive control. Mortality of shrimps was calculated after 24 hrs for blank, positive control and different concentration stock solutions. % Mortality of shrimps was calculated using the following formula (Tibuhwa, 2017) :

$$\% \text{ Mortality} = \frac{\text{Number of dead shrimps x 100\%}}{\text{Total number of shrimps}}$$

Statistical analysis

Statistical analysis was performed by using IBM SPSS version 21. Spearman's Correlation Coefficient Significant (2-tailed) was performed for comparing significant differences between samples for retention of vitamin D_2. Student t-test was performed to compare significant difference between the sample and control for Brine shrimp assays. A significant level of $p<0.01$ (99% significant level) and $p<0.05$ (95% significant level) were applied. Microsoft Excel 2010 was adopted for linear curve fit.

RESULT AND DISCUSSION

Optimization of Vitamin D_2 retention

High performance Liquid Chromatography (HPLC) was adopted for optimizing vitamin D_2 retention from various drying oyster mushroom samples; sundried oyster mushroom (SDOM) powder, cabinet dried oyster mushroom (CDOM) powder, freeze dried oyster mushroom (FDOM) powder and 30 minutes sunlight exposed freeze dried oyster mushroom (SDFD) powder. Standard ergocalciferol of different concentrations were injected, and mean concentration was found at the concentration of 20 µl/ml and retention time (Rt) was at 15.979 minutes. 20 µl/ml was injected

for all the sample, peak area was recorded at the Retention time of 15.979 minutes (as taken from standard ergocaliferol), declining of peak was observed after the specific Rt. The peak area (y) which was obtained from the graph, shown in (Fig.1) concentration (y) of the samples was calculated using $y = 7313x$. The graph in (Fig.1) was constructed in SHIMADZU LabSolutions. (Table 1)shows the observed peak area (y), concentration (x) and vitamin D_2 present in various samples.

Table 1and Fig.2 depicts the peak area, concentration obtained from the graph and vitamin D_2 content in dried oyster mushroom powder per 100 g. Out of four methods of drying, the content of vitamin D_2 was found to be highest in SDOM extract, followed by SDFDOM extract, FDOM extract and lowest in CDOM extract which were 29.44 µg, 26.21µg, 25.52µg and 18.47µg respectively per 100 g of dry sample. Two tailed Spearman's Correlation Coefficient was used for statistical analysis, showing significant at the level of *p* value 0.01 (2 tailed) that means 99% significant. Hypothesis supported by research work done by Nölle *et al.* (2017) in the work, brown button mushrooms were subjected to dry in solar dryer and open sun dry. Studies reported that extracted from solar dried mushroom (39 µg/g d.m) was slightly higher vitamin D_2 content than the extract from sun dried mushroom (36 µg/g d.m) but it was not significant and vitamin D content in mushrooms may be varied due to different species, exposure time, latitude and season. According to Cardwell *et al.* (2018) exposure of fresh button mushroom to sunlight for 15-30 mins could generate abundant amount of vitamin D, which fulfilled daily requirement of vitamin D of many countries. Even though the vitamin D synthesized depend on season, weather, latitude and time of the specific day.

Fig.2 reveals that yield of oyster mushroom after various methods of drying, freeze dried and 30 mins sun dried + freeze dried yielded more than either sun dried or cabinet dried. It also showed that yielding of vitamin D_2 was found to be highest in SDFDOM powder according to the initial weight of the fresh oyster mushroom followed by FDOM, SDOM and CDOM powder extracts. However, the overall content of vitamin D_2 present was highest in SDOM powder extract per 100 g of dried sample.

Toxic analysis by Brine Shrimps Lethality Assay

Brine Shrimps Lethality Assay was performed to analyse toxicity of ethanol extract crude oyster mushroom. 42% of crude extract (0.21g) was yielded after vaporization of ethanol. Table 2 reveals the number of the dead shrimps within 24 hrs in different concentrations and percentage rate of mortality at 24 hrs per each concentration. After monitoring of 24 hours of mortality rate of shrimps, the ethanol extract crude sample was comparatively lesser toxic than $K_2Cr_2O_7$ showing maximum dead of shrimps at higher concentration. As the higher concentration, the number of dead shrimps increased and vice versa. All brine shrimps were found to be mortal after 24 hrs at highest concentration shown in Table 2.Fig.3 &3A show the image of shrimps in different concentration of oyster mushroom extracts.

Median concentration of Lethality (LC_{50}) value was calculated by using percentage (%) mortality of brine shrimps in the different concentrations of sample given in Table2. By using the following formula (Nrior and Gboto, 2017). Study proposed that extract which has the median lethal Concentration (LC_{50}) value is e" 1000 µg/ml (1 mg/ml) is non-toxic (Mai *et al.,* 2024). LC_{50} value of the ethanol extract oyster mushroom was found as 1.0065 mg/ml which signified that the sample was non-toxic while $K_2Cr_2O_7$ was toxic (LC_{50} value - 0.7 mg/ml).

$$LC_{50} = LC_{100} - \frac{\Sigma \text{ dose difference x mean \% mortality}}{\text{\% control}}$$

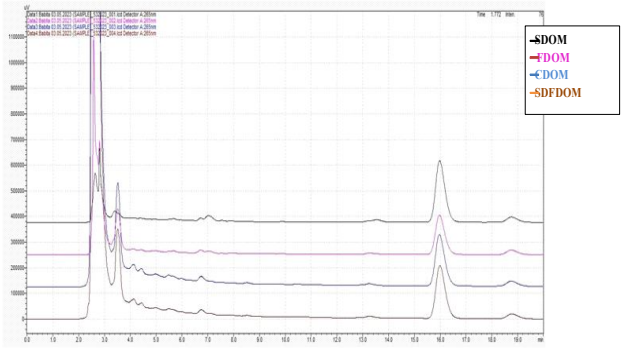

Fig. 1: HPLC chromatogram of various drying methods of oyster mushroom extracts

Table 1: Effect of different drying techniques of oyster mushroom on the impact of vitamin D_2 retention

Samples Name	Area (y)	Conc. (x)µg/ml	Vitamin D_2 content in 1 g of dry sample powder (µg)	Vitamin D_2 content in 100 g of dry sample powder (µg)	Correlation Coefficient Sig. (2-tailed)
Sundried oyster mushroom powder	6276834	858.3117736	0.29	29.44	1.000**
Cabinet dried oyster mushroom powder	3938238	538.5256393	0.18	18.47	.500
Freeze dried oyster mushroom powder	5440614	743.9647204	0.25	25.52	1.000
30 min sunlight exposed freeze dried oyster mushroom powder	5588797	764.2276767	0.26	26.21	1.000**

Injection Volume inal cases- 20 µl; **Correlation is significant the level *p*-value <0.05 (2-tailed);

Table 2: % mortality of Brine shrimps in different concentration of oyster mushroom extract

Sample	Concentration (µg/ml)	Mortality of Brine Shrimp (no. of shrimps dead) (Hour)						LC_{50} Value (mg/ml)	*p*-value
Oyster mushroom (crude extract)		1	2	4	6	24	% Mortality (at 24 hr)		
	100	0	1	4	9	13	43		
	250	1	2	4	10	17	56	1.0065	0.024*
	500	2	2	9	12	20	66		
	1000	2	7	17	21	30	100		
	1500	5	15	21	30	30	100		
Control $K_2 Cr_2 O_7$	1000	30	-	-	-	-	100	0.7	0.374
Blank	Saline water	0	0	0	0	0	0	-	

*Significant level at *p*- value < 0.05

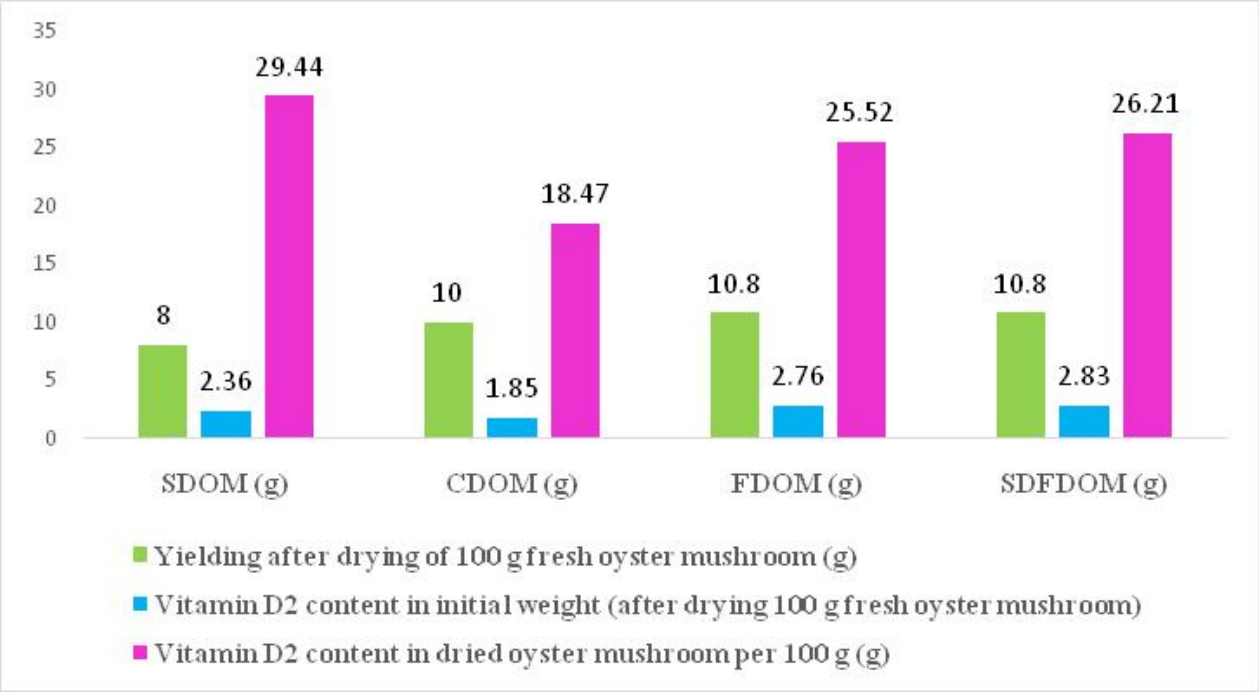

Fig . 2 : Vitamin D_2 content in oyster mushroom from dry sample obtained after various drying techniques of fresh oyster mushroom (100 g) and vitamin D content in initial weight and final weight.

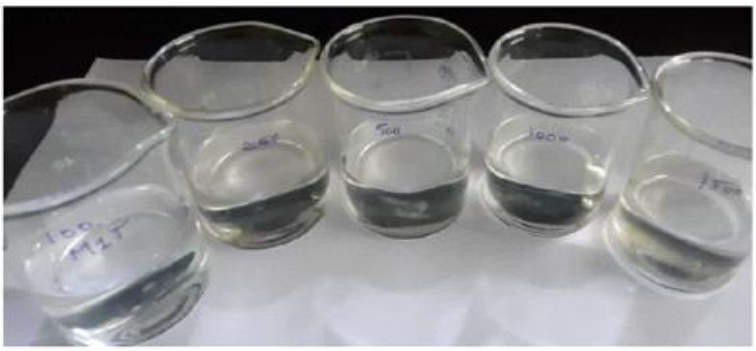

Fig.3 A & B: Crude sample in different concentrations and representative image of Shrimps in the sample respectively

CONCLUSION

The present study demonstrated that the retention of Vitamin D_2 was affected by drying techniques. The extract from sundried oyster mushroom powder was highest in Vitamin D_2 retention followed by 30 mins exposed to sunlight, freeze dried, andcabinet dried. The study also concluded that the ethanol extract oyster mushroom powder was non-toxic when compared to Potassium dichromate as the LC_{50} valuewas >1 mg/ml. Exposure of oyster mushrooms to sunlight improved not only the nutritional values, which may provide a potential source of vitamin D for vegetarians as the sources of vitamin D are limited from plant based foods, but also extended shelf life. Therefore, regular consumption of sunlight exposed oyster mushroom (at least for 30 minutes) might be promising tool to maintain vitamin D status in body.

DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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REFERENCES

Argyropoulos, D., Heindl, A., Müller J. 2011. Assessment of convection, hot air combined with microwave vacuum and freeze drying methods for mushrooms with regard to product quality. *Inter. J. Food Sci. Technol.***46**: 333-342. https://doi.org/10.1111/j.1365-2621.2010.02500.x e

Cardwell, G., Bornman, J. F., James, A. P., Black, L. J. 2018. A review of mushrooms as a potential source of dietary vitamin D. *Nutrients* **10**: 1498.

Hu, D., Chen, W., Li, X., Yue, T., Zhang, Z., Feng, Z., ...& Li, L. 2020. Ultraviolet irradiation increased the concentration of vitamin D2 and decreased the concentration of ergosterol in shiitake mushroom (*Lentinus edodes*) and oyster mushroom (*Pleurotus ostreatus*) powder in ethanol suspension. *ACS Omega* **5**: 7361-7368.

Kalpana, C.A., Babita Devi, N., Ghosh, S., Rashidinejad, A. 2023. Vitamin D. In: *Handbook of Food Bioactive Ingredients* (Eds.S.M.Jafari, A. Rashidinejad, J. Simal-Gandara, J.). Springer, Cham. https://doi.org/10.1007/978-3-030-81404-5_23-2

Mai, N. C., Van, N. T., Hoe, P. T., Giang, V. H., Ban, N. K.,Linh, T. M. 2024. Optimization of brine shrimp lethality test for in vivo toxicity evaluation of poisonous plant species collected from quang tri province. *Academia J. Biol.* **46**: 55-67. https://doi.org/10.15625/2615-9023/18899

Narang, R.,Sharma, A. 2020. Development And Evaluation Of Vitamin-D And Calcium Rich Products Using Different Cooking Techniques. *Plant Archives* **20**: 3687-3690

Nölle, N., Argyropoulos, D., Ambacher, S., Müller, J., Biesalski, H. K. 2017. Vitamin D2 enrichment in mushrooms by natural or artificial UV-light during drying. *LWT-Food Sci. Technol.* **85**: 400-404. https://doi.org/10.1016/j.lwt.2016.11.072

Nongthombam, J., Kumar, A., Ladli, B. G. V. V. S. N., Madhushekhar, M., Patidar, S. 2021. A review on study of growth and cultivation of oyster mushroom. *Plant Cell Biotechnol. Mol. Biol.* **22**: 55-65.

Nrior, R. R.,Gboto, B. 2017. Comparative toxicity of spent mobile phone batteries (Samsung and Tecno) on *Nitrobacter* sp. *J. Environ. Sci. Toxicol. Food Technol.* **11**: 37-44. DOI: 10.9790/2402-1108013744

Patil, J., Ghodke, S., Jain, R.,Dandekar, P.2018. Extraction of vitamin D from button mushroom (*Agaricus bisporus*) using deep eutectic solvent and ultrasonication. *ACS Sustainable Chem. Engineer.* **6**: 10578-10586. https://doi.org/10.1021/acssuschemeng.8b01915

Perduca, M., Destefanis, L., Bovi, M., Galliano, M., Munari, F., Assfalg, M., ...Capaldi, S. 2020. Structure and properties of the oyster mushroom (*Pleurotusostreatus*) lectin. *Glycobiology* **30**: 550-562. https://doi.org/10.1093/glycob/cwaa006

Schill, S., Stessl, B., Meier, N., Tichy, A., Wagner, M., Ludewig, M. 2021. Microbiological Safety and Sensory Quality of Cultivated Mushrooms (*Pleurotus eryngii*, *Pleurotus ostreatus* and *Lentinula edodes*) at Retail Level and Post-Retail Storage. *Foods*.**10**:816. https://doi.org/10.3390/foods10040816

Staffas, A., Nyman, A.,Nordic Committee on Food Analysis. 2003. Determination of cholecalciferol (vitamin D3) in selected foods by liquid chromatography: NMKL collaborative study. *J. AOAC Inter.* **86**: 400-406.

Tibuhwa, D. 2017. Cytotoxicity, antimicrobial and antioxidant activities of *Boletus bicolor*, abasidiomycetes mushroom indigenous to Tanzania. *Tanzania J.Sci.* **43**: 151-163.

Van Hung, N., Fuertes, L. A., Balingbing, C., Paulo Roxas, A., Tala, M.,.....Gummert, M. 2020. Development and performance investigation of an inflatable solar drying technology for oyster mushroom. *Energies* **13**: 4122. https://doi.org/10.3390/en13164122