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EXAMINATION OF VITAMIN D2 CHANGE OF Agaricus bisporus AND Pleurotus ostreatus UNDER THE (UV) B RADIATION

Mertcan Karadeniz[1], Sabri Ünal[1], Temelkan Bakır[2*] & Rifat Sami Sancı[3]
[1]Department of Forest Engineering, Kastamonu University, Kastamonu, 37100, Turkey
[*2]Department of Chemistry, Kastamonu University, Kastamonu, 37100, Turkey
[3]Elit Engineering, 34750, İstanbul, Turkey

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Abstract
Vitamins D are steroid-type vitamins that are soluble in fat, which is naturally found in food, and is responsible for increasing intestinal absorption of minerals such as calcium, magnesium and phosphate.Vitamin D is most commonly found in fatty fish such as salmon, tuna and mackerel, in small quantities in cattle liver, cheese and egg whites.However, it is difficult to obtain enough vitamin D from only natural foods.For this reason, it may be necessary for many people to consume vitamin D-supplemented foods.Some mushrooms contain ergosterol, a variable source of vitamin D2.These substances turn into ergocalciferol (vitamin D2) when exposed to ultraviolet light under controlled conditions.

In this study, ultraviolet (UV) B radiation of 290-320 nanometer wavelength was applied to cultured fungi of *Agricus bisporus* and *Pleurotus ostreatus* containing ergosterol (provitamin D2) for 90 seconds to investigate the changes in vitamin D2 concentrations.For the UV radiation application, a prototype device previously designed by Elit Engineering firm was used.Vitamin D2 values of cultured fungi of *Agricus bisporus* and *Pleurotus ostreatus* were found to be 137 µg / 100 g and 927 µg / 100 g, respectively.As a result, it has been observed that the values obtained are well above the limits set by the European Union Standards (25-50 µg/100g)

Keywords: *Agricus bisporus*,*Pleurotus ostreatus*,Vitamin D2, Ergosterol, UV radiation B.

Introduction
Vitamin D, required for healthy bone and tooth development, increases the absorption of calcium and phosphorus from the gastrointestinal tract. Due to the deficiency of vitamin D which plays a role in the optimal concentration of calcium in the blood, may occur rickets in children and osteoporosis in adult.There are also studies on the association between vitamin D deficiency and the emergence of some diseases such as heart diseases, obesity, diabetes and arthritis (Elbistanlı et al., 2015; Kurt et al.,2015).

The term vitamin D is the general name of a group of steroids that dissolve in fat. Vitamin D has two main sources of cholecalciferol (Vitamin D3) and ergocalciferol (Vitamin D2).Because both vitamin D2 and D3 are metabolized in the same way, they are called a vitamin D by a common name.Both of these provitamins are converted into active vitamin D by the action of ultraviolet rays. Kolekalsiferol (D3) and fungi in animal foods and ergocalciferol (D2) in plant foods can be taken with diet. D3 is the form that humans and animals naturally take from the sun through the skin. Vitamin D2 is a herbal vitamin and ergosterol in the fungus transforms into D2 Vitamin (ergocalciferol) in contact with UV-B. It has been found to be abundant especially in fatty fish such as salmon, mackerel, fish liver oil, egg yolk, dairy products and wild mushrooms(Wu and Ahn, 2014).

Agaricus bisporus and *Pleurotus ostreatus* preferred by the people because of its flavor and these mushrooms(Bakır et al., 2017). Mushrooms that containing 90% water are a good source of vitamins and proteins. Contains vitamin B and vitamin C complexes such as thiamine, riboflavin and niacin. Mushrooms are rich in mineral substances like K, P and Na.It contains higher amounts of protein (20-35% in dry matter) than many vegetables and fruits.They also contain vitamin D precursor, ergosterol, which is present in some yeasts

and fungi, is transformed into vitamin D2 due to the effects of ultraviolet heat. As a result of this information, this study aims to meet the daily vitamin D needs of our people by enriching the vitamin D2 in fresh mushrooms by applying ultraviolet rays.

Material and Method

In this study, we were used *Pleurotus ostreatus* which produced in Kastamonu University Mushroom Research and Application Center and *Agaricus bisporus* that obtained from market.D2 vitamins enrichment in the mushrooms by using artificial light was made using the UV-B cabinet system that designed (Figures 1).

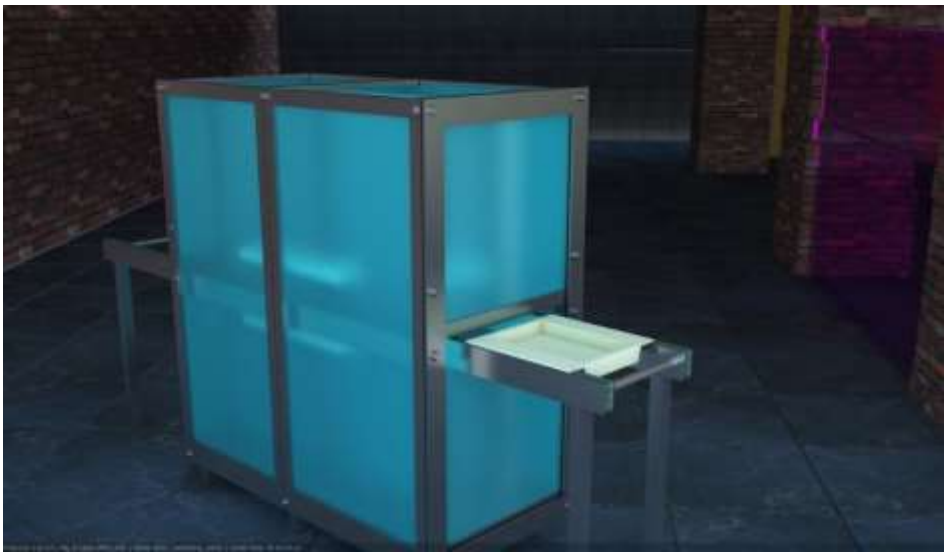

Figure 1. Overview of the designed system

Although our system is a safe one, UV-B lamps are constantly burning throughout the production.The UV-B lamps are protected by a cabinet made of a UV-B wavelength-free material. The UV-B lamps are approached to the nearest distance to the mushrooms to achieve a high UV-B value.In addition, this system is designed to be suitable for commercial production so it is produced with conveyor belt.

D2 vitamin Enrichment process in the mushroom was designed with the help of 3 meter conveyor specially designed by us. In the study, first of all, the broadband Philips UV-B medical lamps were used, and the narrow-band UV-B lamps were also tested. Wavelengths of UV-B lamps range from 290 nm to 315 nm. Although the peak value of UV-B lamps in broadband is 302nm, the narrow band has a wavelength of 311nm.The mushroom trays were 405x600x3mm and were made of acrylic plate. Acrylic sheets pass the UV-B light between 290-315 nm at 85% - 90% rate.Each tray take 2.5 kg mushrooms.

The UV-B intensity of the TL Philips UV-B Broadband TL 20W / 12 ref bulb with 6 reflectors to be placed 6 cm above the cork inside the UV-B enclosure is calculated as 18,37 w / m². 6 Philips UV-B Broadband TL 20W / 12 B bulbs will be placed 3 cm below the acrylic tray and 36.75 w / m². UV-B wavelength exceeding 6 cm from the tray, while the protective UVT plate passed 12% of the value of 16,17 w / m², while the bottom of the cork tray UV-B, acrylic plate passes through the average approximately 12% and 32.34 w / m².The total intensity of the upper and lower lamps is 48.51 w / m². The total value was increased to about 73 w / m² with a polished aluminum reflector to be placed behind the UV-B lamps. This value is about 18 times the summer sun in Turkey.The values we are trying to compute are the maximum theoretical performance values calculated from the distance we measured from the Autocad drawing.

UV-B energy and density of the lamps in the closed dosing cabinet were measured periodically with HD2102.2 Delta Ohm Light Intensity Measurement and Recorder and the decreasing UV-B densities of the lamps were

observed and daily dosing times were calculated according to the results.The UV-B measuring probe can measure the UV-B intensity of 0,0001w / m²-20000w / m².This device measures UV-B waves in the range of 285-315 nm and has a peak value of 305 nm.

UV-B dosing was done in 2 ways;

1. When conveyor belt is in stop position,
2. While conveyor belt is in motion with speed control.

In the stop position of the conveyor belt, UV-B energy measurements were made by HD2102.2 Delta Ohm with 1 meter extension cable and by wireless UVICURE PLUS II radiometer in motion.Mushrooms were initially given 30 min, 1, 2 and 3 minutes dosing times to give D2 Vitamin (µg / 1000g) analysis. 600IU / 100g D2 Vitamin needed to obtain the required times were calculated.In addition, during the production process of Vitamin D2, temperature, humidity and all or sliced conditions of the mushroom were taken into consideration.

UV-B Application on*P. ostreatus* and*A. bisporus*

In general, the production of commercial vitamin D2 is done by Pulse UV-B system (Ko et al., 2008; Kalaras, 2012). 600 IU / 100 g is obtained with1-3 pulse in a second.In this system the mushrooms are exposed to unilateral UV-B. Since the lower part of the tray is not exposed to light, it does not form Vitamin D2. Only 600 IU / 100 g of total fungus in the storage container is captured.It is working with very high voltage and the temperature of the lamp is up to 50 ^{0}C. Therefore, in order to reduce the temperature, blower and exhaust fan must be installed on the machine. The UV-B pulse system is delivered with a UV-B dose of approximately 150 and a maximum of 2800 times the amount of sunlight.

In the study, UV-B rays (285-315 nm wavelength) was applied to the mushrooms on both sides at the same time. The mushrooms were placed whole or sliced into trays made of plexiglass, which passed 88% of the UV-B wave lengths.The mushrooms were placed as whole or sliced into trays made of plexiglass. A total of 12 (over 6 trays, 6 trays on top of the trays) of the trays were subjected to UV-B wavelengths with 20w UV-B wide band medical lamp.All the surfaces of the mushrooms visible from the top and bottom in the tray are in contact with UV-B;

- Increase in the amount of vitamin D2 (ergocalciferol) provided,
- Homogeneous distribution of vitamin D2 provided in mushrooms and
- Vitamin D2 formation time was shortened.

Preparation of vitamin D2-rich mushroom extract:

The lyophilized mushroom samples were treated with sodium ascorbate solution, ethanol and potassium hydroxide (50%) solution under reflux at 80°C for 1 hour.The mixture was taken up in a separatory funnel after cooling and subjected to liquid-liquid extraction with distilled water followed by ethanol and n-pentane.The collected organic layers were washed with diluted KOH solution and diluted ethanol.The extract was then obtained by evoporated on the rotavapor (Janakakumara, 2005; Jasinghe and Perera, 2006).

HPLC analyzes were performed in Eurofins research laboratories using Hypersil Gold column (100x2.1).The method used was based on literature information and was modified by us in the most appropriate way.The standard vitaminD2 was quantified with the aid of a scale curve which would be plotted on the basis of the peak areas of the spectrum, which were shown at specific concentrations (George and Szczesniewski, 2009).

Results

Vitamin D2 (Ergocalciferol) in edible mushrooms is caused by the exposure of ergosterol in the fungus to severe UV-B wavelength (280-315 nm).UV-B wavelength can be obtained from sun and artificial light.The unit of ergocalciferol (D2 Vit.) Is µg/g, and 1 µg/g equal to 40 IU vitamin D2.According to the World Health Organization, the amount of vitamin D2 that should be taken daily by adult people is 600 IU/100g.The UV-B wave length of the sun increases by 10% every 1000 meters, while the artificial light has a very different value from 10 cm. For example, the Philips UV-B Broadband TL 20W / 12 m lamp was measured as 11.3 w/m² at

0.10 m (10 cm) and 22.9 w/m² at 0.05 (5 cm) perpendicular to the object.UV-B irradiation with artificial light, this feature is taken into account in the UV-B cabinet lamp distance settings are made.The UV-B lamps used do not produce ozone gas. Therefore, it is not necessary to clean the air inside the cabinet. In our application, UVT (Ultraviolet Transmitter) plates were used because of the economics and wide range of usage areas.

UVB curing conveyor was manufactured and the first attempt was made by taking the dose values in the literature. The following values were obtained from the initial analysis values (Table 1).

Table 1.Ergocalciferol values obtained in P. ostreatus and A. bisporus mushroom species by applying a dose of 0.348j / cm²

Mushroom	Ergocalciferol (Vitamin D2) Value (µg/100 g)	Ergocalciferol (Vitamin D2) Value (IU/100 g)	U(%)
Pleurotus ostreatus	927	37.080	26
Agaricus bisporus	137	5.480	26

These values should be 400 IU/100g (World Health Organization) or 600 IU/100g (European Food Safety Authority). According to the Turkish food codex, the amount required for vitamin D to be a supplementary food is 200IU / 100g.The values above were taken in 90 seconds. Analysis results showed that the time required to reduce the duration of the fungus from the time of the lamp to 90 L, 600IU / 100g vitamin D2 to reduce the time to 10-35 seconds. *Agaricus bisporus* (1.6 kg) and*Pleurotus ostreatus* (0.7 kg) were enriched 600IU / 100g with vitamins D2 in every 10-35 seconds.

Conclusion

The severity of the sun is not sufficient to synthesize the necessary amount (1000 IU) of vitamin D2 per day for adult humans in the winter months. For this reason people should do vitamin D supplementation by diet.In developed countries, many foods such as milk and milk products, mushrooms, margarines and fruit juices are sold with the addition of vitamin D.This enrichment practice has become an important health policy to prevent vitamin D deficiency.

This study has shown that UV-B applications increase vitamin D2 in the mushrooms.In addition, these mushrooms can be pulverized and used as food supplements for humans and animals.Thus, as a result of the study, added value can be obtained in the livestock sector (meat and milk livestock, aquaculture, etc.) as well as human diet.

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References

[1] Bakır, T., Ünal, S., Karadeniz, M., & Bakır, A. S. (2015). A Comparative Study On Antioxidant Properties And Metal Contents Of Some Edible Mushroom Samples From Kastamonu, Turkey. Journal of Food and Health Science, 3(4), 132-140.

[2] Elbistanlı, M.S., Çelik,M., Güneş, S., Yegin,Y., Kaya,K.H., Canpolat, S., Kayhan,F.T. (2015). Relationship between upper respiratory tract infection incidence and low vitamin D levels in children, KBB Uygulamaları, 3(2):51-55. doi: 10.5606/kbbu.2015.42713

[3] George M.P.,Szczesniewski A., (2009). Rapid Analysis of Vitamin D in serum usingtriplequadrupole LC-MS, Agilent Technologies, Inc., USA

[4] Janakakumara,J. V.,(2005). Conversion of Ergosterol Edible Mushrooms to Vitamin D2 By UvIr radiatıon. PhD Thesis. National University of Singapore,Food Science and Technology Programme Department of Chemistry,Republic of Singapore, 2005, 137 pages

[5] Jasinghe, V.J.,Perera C.O., (2006). Ultraviolet irradiation: The generator of Vitamin D2 in edible mushrooms. Food Chemistry, Volume 95, Issue 4, April 2006, Pages 638–643

[6] Kalaras, M. D., (2012). Production of Ergocalciferol (Vitamin D2) and Related Sterols in Mushrooms With Exposure to Pulsed Ultraviolet Light. PhD Thesis.The Pennsylvania State University The Graduate School Department of FoodScience, USA, 2012, 115 pages

[7] Ko, J.A., Lee, B.H., Lee, J.S., Park, H.J., (2008). Effect of UV-B Exposure on the Concentration of Vitamin D2 in Sliced Shiitake Mushroom (Lentinus edodes) and White Button Mushroom (Agaricus bisporus). J. Agric. FoodChem. 2008, 56, 3671–3674

[8] Kurt,E.E., Koçak F.A., Erdem, H. R., Tuncay, F., Kıranatlıoğlu, F. (2015). Kırsal Bölgede D Vitamini Farkındalığının Değerlendirilmesi: Kesitsel Bir Çalışma. Journal of Physical Medicine & Rehabilitation Sciences/Fiziksel Tup ve Rehabilitasyon Bilimleri Dergisi, Cilt 18,Sayı 3.

[9] Wu W.J., Ahn B.Y. (2014). Statistical Optimization of Ultraviolet radiate Conditions for Vitamin D2 Synthesis in Oyster Mushrooms (Pleurotus ostreatus) Using Response Surface Methodology. PLoS ONE 9(4): e95359. doi:10.1371/journal.pone.0095359.