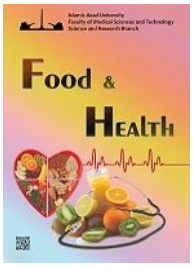

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Effect of different drying methods on Vitamin D content in *Agaricus bisporus* Mushroom

Anousheh Sharifan[1*], Mina Kouhi[1], Qobad Asgarijafarabadi[2]

[1] Department of Food Science and Technology, Islamic Azad University, Science and Research Branch, Tehran, Iran
[2] Department of Agriculture, Islamic Azad University, Pishva-Varamin, Iran

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ABSTRACT

Vitamin D is a significant nutrient in a human's diet and its deficiency has become a noticeable problem in the world. This study tried to evaluate the effective factors in preserving vitamin D in dried mushrooms using sunlight and ultraviolet light (UV-C). The effect of irradiation on vitamin D_2 and D_3 in *Agaricus bisporus* mushroom was investigated. In this study, mushrooms were cut (in 5 mm) and dried in two ways of hot air and freeze-drying at 60 °C for 60min and -50 °C for 24 h respectively, then powdered and after that, the powders were exposed to the sunlight and UV for 5, 15 and 30 min and finally the amount of vitamin D was measured by HPLC spectrometer. The highest vitamin D_2 content was observed in a mushroom powder dried by freeze dryer and exposed to sunlight for 5 min and also the one which was irradiated with UV for 30 min. The highest vitamin D_3 content was observed in a mushroom powder dried by the hot dryer and exposed to sunlight for 30 min and the mushroom powder dried by freeze dryer and then irradiated with UV for 30 min. Applying ultraviolet light and sunlight in dried mushrooms increased vitamin D levels. The results of this study showed that the drying methods of mushroom were effective in maintaining and increasing the amount of vitamin D_3 in the foodstuff.

1. Introduction

Vitamin D_2 was discovered by Edward Melanbay in 1912 as the only vitamin that would be produced in the presence of ultraviolet rays of the sun (1). The white *Agaricus bisporus* mushroom is an important source of protein, phosphorus, magnesium, and vitamin D. It is necessary to mention that ergosterol in mushroom converts to vitamin D in our body after consumption. It is a sterol that can make vitamin D after exposure to the sunlight for 20-30 min (2). Vitamin D synthesis in the body is associated with some factors such as latitude, season, exposure time, direct sunlight, skin color, and age (3). On the other hand, exposure to sunlight for a long time may cause skin cancer (4). Ergosterol changes in the liver to 25-hydroxy vitamin D and then to O, 25-hydroxy vitamin D hydroxide in the kidneys and increased the level of vitamin D in human blood serum, therefore mushroom is a good source of vitamin D. It is suggested to intake 2000 IU of vitamin D (5, 6). Also, the ergocalciferol and ergosterol in mushrooms would change to vitamin D after exposure to sunlight and ultraviolet radiation. Increasing the ultraviolet rays leads to an increase in the amount of vitamin D in mushroom (7). Ultraviolet radiation is classified by the International Commission on Radiation as the following: (a): 315-400 nm (UV-A): in sun rays and electric lights, can't be absorbed by the ozone layer and also cause skin wrinkling; (b): 290-315 nm (UV-B): in the form of a mercury lamp, it would be absorbed by the ozone layer in large amounts; (c): 190-290 nm (UV-C): in the mercury arc, it has antimicrobial properties and would be completely absorbed by the ozone layer (8, 9). To investigate the process of increasing vitamin D in mushrooms, the *Agaricus bisporus* has used in an animal model (laboratory rats). In the first step, 30 mice were fed with a diet without vitamin D for a week, then their bone density was analyzed. The mice were divided into 12 groups and the first group was feed with UV-irradiated mushrooms for 4 weeks and the second group was normally fed. The level of 25-hydroxy vitamin D in the first group was about 22 nmol/l and in the

*Corresponding author: Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail address: anousheh.sharifan@yahoo.com (Anousheh Sharifan).

second group 1.09 nmol/l. The bone density of the first group was significantly higher than the second group ($p<0.01$). The results showed that vitamin D was better absorbed in the UV-irradiated mushrooms by the animal's system (10). In this study, the amounts of vitamin D in dried mushroom powder (hot-air and freeze-dried) treated by sunlight and UV for 5, 15, and 30 min were investigated.

2. Materials and methods

2.1. Materials

Button *Agaricus bisporus* mushrooms were purchased from Golbarg mushroom company in Iran. All chemicals used in this study were purchased from Merck (Darmstadt, Germany) and the microbial culture medium of DRBC (Sigma, USA), Medical Ethanol (96%) was prepared from Bidestan Company, Iran.

2.2. Irradiation methods

2.2.1.Ultra-violet (UV-C) lamp

In this project, UV-C (Osram-HNS-OFR-Made in Japan. 32U7) was used with a wavelength of 190-290 nm that consists of 2 cylindrical tubes with a blue luminaire lamp. The lamp is 90 cm, 30 watts, and placed in a special wooden chamber. The distance of samples and lamp is adjustable and it was about 30 cm in this study. The 14 samples of mushroom powder were individually dried by the hot-air dryer and freeze-dryer and then treated under UV rays for 5, 15, and 30 min.

2.2.2.The Sunlight

The sunlight's UV ray is ranged from 0 to 10. This study was done in winter and the highest amount of sun UV ray in this season is 3 out of 10. The 14 samples of powdered mushroom were individually dried by hot-air and freeze-dryer and then each sample was exposed to the sunlight for 5, 15, and 30 min. Finally, the powdered mushrooms were packed in plastic bags and applied for vitamin D analysis.

2.3. The effect of different parameters on vitamin D content

The mushrooms were selected randomly and white Button Mushrooms *Agaricus bisporus* were cut in 5 mm and placed in a stainless-steel tray and put in an oven with an optimum temperature of 60˚C for 60 min and the moisture content was measured. After that, the dried mushrooms were powdered (with Moulinex-Modle deposed, made in France). Then the samples were exposed to the sunlight (UV=3 out of 10) for 5, 15, and 30 min. Similarly, the mushrooms were cut in 5 mm and initially froze in a freezer of -20˚C for 24 h and then dried in a freezer with a temperature of -50˚ for 24 h. After all the powdered samples were exposed to the UV-C lamp with a wavelength of 190-290 nm and the sunlight. Finally, vitamin D was measured.

2.4. Chemical analysis

2.4.1.The moisture content

The moisture content was measured according to NIS No. 672 by the following equation (11):

$$Moisture\ content = (M_1 - M_2 / M_1 - M_0)$$

where, M_0 was the weight of the empty plate (g), M_1 was the weight of the empty plate before drying (g); and M_2 was the weight of the empty plate after drying (g).

2.4.2.The ash content

The amount of ash was measured according to INS No. 1197 according to the following equation (12):

$$Ash\ content = (M_3 - M_1 / M_2 - M_1 \times 100)$$

where, M_1 was empty crucible, M_2 was crucible and sample, and M_3 was crucible and the ash weight (g).

2.5. The vitamin D measuring by HPLC

The vitamin D was measured according to NIS No. 13579. Freeze-dried mushroom powders were mixed with potassium hydroxide (85%) and were saponified for 1 h, the acetonitrile/methanol 75:25 was used as the solvent. Then the organic layer was put into a round-bottom flask, rotary-evaporated (Heidolph, Labarota 4003 control-Heidolph Persia, Research made easy) and immediately re-dissolved in 5 ml ethanol. Finally, the samples were passed through a filter and the residue was dissolved and the samples were injected to HPLC using acetonitrile/methanol. The ergosterol, vitamin D_2, and D_3 are evaluated by detecting the UV absorption at 282 nm. Each sample was injected at a flow rate of 2.3 ml/min and 30 min and the most suitable spike resolution was chosen (9). It should be noted that vitamin D_3 was used as the Standard because it would not interfere with other components (14). The other advantages are the ability to be identified in the peak resolution at 282 nm. The initially Standard solutions of vitamin D_3 (10, 50, and 100 ppm) were prepared and then 20 µl of the samples were injected into the chromatography system (9). The linear range was tested and controlled according to the determined concentrations. The filtered sample (20 µl) was injected into a (UV detector using HPLC, C-R5A, CHROMATOPAC, Kern, Australia), and with an acetonitrile/methanol as a mobile phase at a flow rate of 2.3 ml/min with a UV detector at 282 nm. Vitamin D was analyzed by comparing the retention times of standards and the amount was measured using a calibration curve.

2.6. Statistical analysis

The effect of irradiation method (UV and sunlight) in 3 levels (5, 10, and 15) and the type of drying (hot air and freeze)

were investigated in a factorial experiment in a completely randomized design by the Duncan method, all analyzes were performed using SPSS statistical software.

3. Results and discussion

The chemical analysis of mushroom in different drying methods is shown in Table 1.

Table 1. The moisture and ash content of mushroom in different drying method.

Mushroom	Drying method	Moisture percent	Ash (%)
Fresh mushroom	-	90.4	328.125
Powdered mushroom	Hot-air-dryer	6.85	379.88
Powdered mushroom	Freeze dryer	3.08	7

In this study, the effect of the variables on the amount of vitamin D_2 and D_3 was investigated. The results showed that the drying methods had effects on vitamin D_2 ($p<0.0001$) and D_3 ($p<0.006$) content. According to the results (Fig. 1-4), the control sample which was dried with a hot-air dryer affected vitamin D_3 content ($p<0.0001$). The 3 dried treatments with hot-air and freeze dryers, that were irradiated for 5, 15, and 30 min with sunlight, showed a more significant effect on vitamin D_3 content ($p<0.0001$).

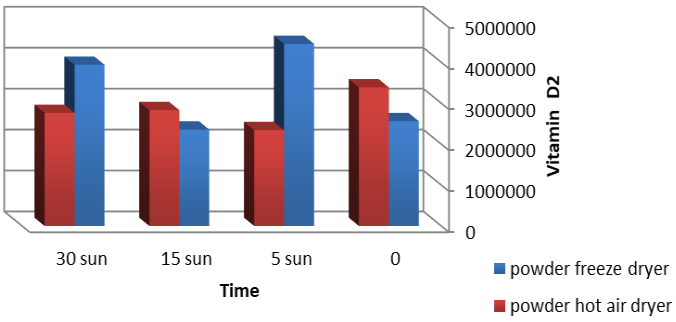

Fig. 1. Vitamin D_2 in freeze-dried powder and hot-air dried samples treated by sunlight.

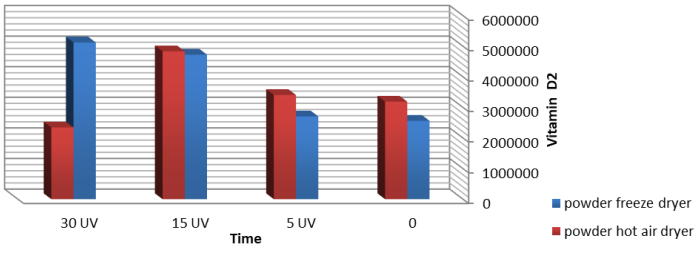

Fig. 2. Vitamin D_2 in freeze-dried powder and hot-air dried samples treated by UV-C.

however, the 3 dried treatments with hot-air dryer and irradiated for 5, 15, and 30 min with UV(UV-C), affected vitamin D_3 ($p<0.0001$). The highest vitamin D_2 content was observed in the treatment dried by freeze dryer and exposed to sunlight for 5 min (Fig. 1) and the one which was irradiated with UV for 30 min (Fig. 2).

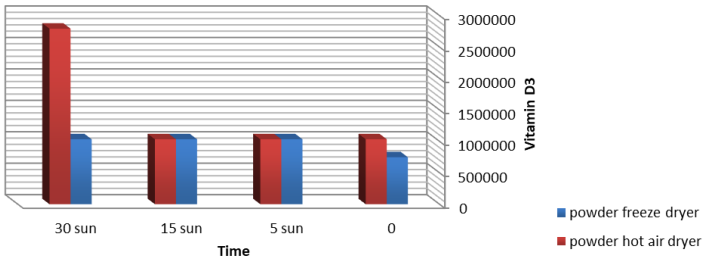

Fig .3. Vitamin D_3 in freeze-dried powder and hot-air dried samples treated by sunlight.

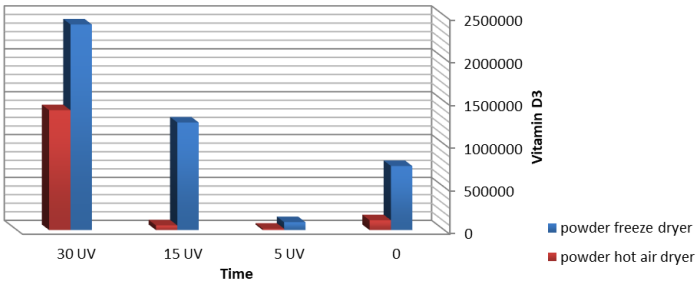

Fig. 4. Vitamin D_3 in freeze-dried powder and hot-air dried samples treated by UV-C.

The highest vitamin D_3 content was observed in the treatments dried by the hot dryer and exposed to sunlight for 30 min (Fig.3) and the one dried by freeze dryer and irradiated with UV for 30 min (Fig.4). In this study, drying and irradiation of button mushrooms would increase the amount of vitamin D_2 and D_3. Hung et al (2015), also showed that vitamin D_2 levels in button mushroom increased to 208 µg/g after ultraviolet radiation (15). In 2008, some researchers applied the high-intensity pulsed ultraviolet radiation at the wavelength of 100 to 800 nm for converting ergosterol to vitamin D_2 at a lower rate compared to the continuous radiation (16). Another research has shown that using ultraviolet pulse beams group B was more effective in increasing the vitamin D_2 content in the white mushroom *Agaricus bisporus* in comparison to group C (17).

4. Conclusion

The results of this study, showed that the drying methods was effective in maintaining and increasing the amount of vitamin D_3 in the dried mushroom. The dried mushroom powder with hot-air and freeze dryers under ultraviolet radiation and sunlight has been affecting the preservation and enhancement of vitamin D_3 in dried button mushroom. It should be noted that dried treatments with hot-air dryer exposed to ultraviolet (UV-C) radiation was better on preserving and increasing the amount of vitamin D_3 in dried mushroom.

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*Corresponding author: Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail address: anousheh.sharifan@yahoo.com (Anousheh Sharifan).