Optimization of UV irradiation conditions for the vitamin D₂-fortified shiitake mushroom (*Lentinula edodes*) using response surface methodology

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Abstract The aim of this study was to investigate the optimal conditions to process vitamin D₂-fortified shiitake mushrooms through UV irradiation for industrial processing. Response surface methodology was used to identify the optimum conditions of the following highly influential factors on vitamin D₂ synthesis: temperature, UV dosage, and moisture content. The optimal conditions of those variables were 40.56 °C, 36.27 kJ/m², and 80.46%, respectively, and UV dosage was the most effective variable. The amount of vitamin D₂ obtained under the optimal conditions was 29.87 ± 1.38 µg/g (dry mass: DM) which was well matched with the predicted value of 32.33 µg/g DM. The effects on the texture and color of shiitake mushrooms were also evaluated after the fortification process under the optimal conditions, and no adverse effects were observed compared to those of fresh shiitake mushrooms.

Keywords Vitamin D₂ · UV irradiation · Shiitake mushroom · Response surface methodology

Introduction

Vitamin D has two major forms—vitamin D₃ and vitamin D₂. Vitamin D₃ is produced in animal skin, whereas vitamin D₂ is made from fungi or mushrooms that contain ergosterol, and both conversion mechanisms are triggered by ultraviolet (UV) irradiation. Vitamin D acts as a hormone, and its main role is to maintain calcium homeostasis, retention, and bone deposition of calcium and phosphorus [1]. Role and mechanisms of vitamin D toward autoimmunity, anticancer actions, and prevention of hypertension are also reported [2].

Vitamin D deficiency is a disorder that is common worldwide [3]. It is known that insufficient vitamin D may lower calcium absorption and elevate parathyroid hormone levels that could finally worsen bone loss [4]. Moreover rickets, secondary hyperparathyroidism, osteoporosis, and osteomalacia could be caused in the adult and senior population who are in severe vitamin D deficient state [5].

Oral intake of vitamin D through food may overcome this deficiency. However, very few foods naturally contain vitamin D—these include oily fish such as salmon, mackerel, and herring or fish liver oil [2]. Mushrooms are known to be the only non-animal-based food available for vegans to provide vitamin D [6]. Although wild or cultivated mushrooms are rich in the precursor of vitamin D₂ (ergosterol), they lack vitamin D₂ itself [3]. Therefore, artificial conversion of ergosterol to vitamin D₂ using UV rays is essentially required to make mushrooms good sources of vitamin D₂.

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Shiitake mushroom is the second most commonly produced edible mushroom worldwide [7]. It is reported that shiitake mushrooms contain relatively higher content of ergosterol compared to other mushroom strains and produce higher vitamin D₂ under same UV irradiation conditions [3]. The selective apoptotic properties of ethanol extract of shiitake mushroom toward murine skin carcinoma cells were reported [8], and increased antioxidative properties of aqueous extracts from UV-B-treated Shiitake mushrooms were reported [9].

Concrete and practical conditions that can improve vitamin D₂ levels of mushrooms without causing adverse effects on their edible qualities are required for commercialization. Several studies trying to increase vitamin D₂ content of mushrooms have been reported. However, some studies reported long UV exposure time (i.e. 1 or 2 h) [10, 11], that could be impractical to increase vitamin D₂ of mushrooms. Other studies also tried to obtain optimal conditions by a one-factor-at-a-time method [3, 12, 13], which has limits to reflect concurrent effects of variables [14]. Different states of mushrooms (e.g. mushroom strains, moisture content of mushrooms, freshness) and uncontrolled UV irradiation conditions (e.g. ambient temperature and overall UV dosage) also could become hurdles for industries and mushroom growers to produce vitamin D₂-fortified mushrooms commercially.

To obtain optimal conditions for effective vitamin D₂ production, response surface methodology (RSM) would be a useful statistical tool. RSM has been widely used for optimization in the field of biochemistry as well as in food processing [15, 16]. To the best of our knowledge, few studies have focused on establishing the optimal conditions for vitamin D₂ fortification using RSM. The main idea of this study was to use RSM to determine the optimal production conditions (temperature, UV dosage, and moisture content) for effective vitamin D₂-fortification in shiitake mushrooms through UV irradiation. Changes in the textural and color properties of shiitake mushrooms after vitamin D₂-fortification under optimum conditions were also studied.

Materials and methods

Mushrooms and chemicals

Freshly cultivated shiitake mushrooms (*Lentinula. edodes*) from the Yang-pyeong district, Korea, were purchased from a local supermarket and were used immediately in the experiments. Fresh mushrooms that had pilei with a diameter ranging from 5 to 6 cm were chosen as samples. The mushrooms that had damaged portions were excluded and the whole pileus part of each shiitake mushroom was

used for this study. Highly concentrated UV-B lamps (PL-L, Philips, Poland) were used for UV irradiation. A photoradiometer (DELTA-OHM, Italy) was used to evaluate UV dosage. Vitamin D₂ standards were purchased from Sigma-Aldrich (Bellefonte, PA, USA). L-ascorbic acid (99.6%) and potassium hydroxide (85.0%) were purchased from Junsei Chemical Co. (Japan). HPLC grade methanol, ethanol, acetonitrile, and n-hexane were purchased from Duksan Pure Chemical Co. (Korea).

Preliminary test

A preliminary test was designed to determine the desirable ranges of the three independent variables—air temperature during UV irradiation, overall UV dosage, and moisture content of mushroom samples. The first step of the preliminary test was conducted to determine temperature range, which was varied from 30 to 45 °C, using raw shiitake mushrooms, under a UV dosage of 30 kJ/m². The next step was to investigate UV dosage, using a range from 10 to 50 kJ/m², with raw shiitake mushrooms at 35 °C. The last step was performed to study the effect of moisture content on vitamin D₂ production. The expected drying time to control moisture content of shiitake mushroom samples was calculated before UV irradiation. The moisture content of a batch of mushrooms (n = 20) was controlled by using a drying oven (80 °C). The moisture content of samples after drying was recorded using a moisture analyzer (MX-50, A&D company Ltd., Japan). The residual moisture content of mushroom samples was calculated by measuring weight loss of the mushrooms using an analytical balance every 30 min. The samples were dried until a moisture content of 70 to 87% (wet base) was obtained. After controlling the moisture content, UV irradiation was performed at 35 °C and 30 kJ/m².

Experimental design for response surface methodology

RSM was performed to optimize the conditions for vitamin D₂ enrichment from shiitake mushrooms irradiated with UV. Temperature (X_1 , °C), UV dosage (X_2 , kJ/m²), and moisture content (X_3 , %) were set as independent variables. Based on the results of the preliminary tests, the coded value and the corresponding actual value of the three independent variables are provided in Table 1. After setting the ranges of the three parameters, the expected drying time to control moisture content of samples was calculated prior to RSM trials. The equation for expected drying time for controlling moisture content was acquired as follows:

$$Y = -0.11X + 84.62 \quad (X < 180)$$

Table 1 Experimental runs and responses

Design point	Factors ^a			Response (Y)
	X ₁	X ₂	X ₃	vit D ₂ content (μg/g)
1	− 1 (30)	− 1 (20)	− 1 (70)	20.28 ± 0.42
2	− 1 (30)	− 1 (20)	1 (80)	20.62 ± 0.6
3	− 1 (30)	1 (40)	− 1 (70)	26.98 ± 1.03
4	− 1 (30)	1 (40)	1 (80)	27.7 ± 0.66
5	1 (40)	− 1 (20)	− 1 (70)	24.7 ± 0.82
6	1 (40)	− 1 (20)	1 (80)	29.97 ± 0.82
7	1 (40)	1 (40)	− 1 (70)	26.32 ± 0.95
8	1 (40)	1 (40)	1 (80)	32.16 ± 1.79
9	0 (35)	0 (30)	0 (75)	26.77 ± 1.22
10	0 (35)	0 (30)	0 (75)	29.9 ± 1.47
11	− 1.682 (26.59)	0 (30)	0 (75)	24.45 ± 0.76
12	1.682 (43.41)	0 (30)	0 (75)	28.98 ± 0.23
13	0 (35)	− 1.682 (13.18)	0 (75)	21.32 ± 1.74
14	0 (35)	1.682 (46.82)	0 (75)	32.14 ± 0.87
15	0 (35)	0 (30)	− 1.682 (66.6)	23.45 ± 2.77
16	0 (35)	0 (30)	1.682 (83.4)	28.47 ± 1.06

^aFactors: temperature (X₁), UV dosage (X₂), and moisture content (X₃)

where Y stands for moisture content (%) of mushroom samples and X for drying time (min). The relationship between drying time and moisture content of mushrooms were closely correlated, with a high value of coefficient of determination ($R^2 = 0.9875$).

Sixteen batches of mushroom samples (six mushrooms for each batch) were prepared for RSM trials. After controlling the moisture content of the samples, UV irradiation was performed in a temperature-controllable UV incubator. Mushroom samples were separated from each other horizontally, on a plastic tray, and placed under the UV-B lamp at a distance of 12 cm, which made the intensity of the lamp 17.5 ± 0.76 W/m² in an UV incubator. The gill side of every mushroom sample was placed facing the lamp.

A central composite rotatable design (CCRD) was used to fit a second-order model for the three variables at five levels. The CCRD design consisted of 16 experimental points with 8 vertex points, 6 axial points, and 2 replications of center points. The following quadratic polynomial equation was fitted using the data in Table 1:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j$$

where Y denotes the dependent variable of vitamin D₂ content, β_0 is a constant, and β_i , β_{ii} , and β_{ij} are regression coefficients. X_i and X_j are levels of the independent variable. The samples for RSM analysis were prepared following Table 1. The equations represent the linear, quadratic, and interaction effects of the variables.

Analysis of vitamin D₂

Vitamin D₂ from raw and treated mushroom samples was extracted and analyzed based on the method by Ko et al. [6], with slight modifications. Lyophilized mushroom sample powder (1 g) was accurately weighed and mixed with 1 g of L-ascorbic acid into a 250-mL round-bottom flask, followed by the addition of 50 mL of ethanol (95%) and 25 mL of 50% potassium hydroxide. The mixture was saponified under reflux at 85 °C for 30 min. The extraction was performed using 30 mL of *n*-hexane and 10 mL of de-ionized water. The aqueous layer was removed and the same procedure was repeated twice. The remaining pooled organic layers were washed with de-ionized water until neutralized. The *n*-hexane layer was transferred to a round flask, rotary evaporated at 50 °C until dry and re-dissolved with 2 mL of a mixture solution (acetonitrile:methanol:isopropyl alcohol = 3:1:2). After collecting vitamin D₂, the solution was filtered (0.45-μm pore size PTFE membrane, Whatman International Ltd.). An 8.5-μL filtered sample was injected with an autosampler into the HPLC system (Waters 2690, Waters Corp.), which was equipped with a 996-photodiode array detector (Waters Corp.). The reverse phase column (Polaris C 18-A, 3 μm, 3.0 × 250 mm, Agilent, Netherlands) was used for vitamin D₂ quantification. The mobile phase consisted of methanol/ acetonitrile (25:75, v/v), at a flow rate of 0.43 mL/min. The wavelength for UV detection was 265 nm, and the temperature setting for the column was 25 °C.

Limit of detection (LOD) and limit of quantification (LOQ) for vitamin D₂ were evaluated by determining the average concentrations that gives the signal three times the background noise and ten times the background noise, respectively. LOD was 0.2 µL/mL and LOQ was 0.6 µL/mL. Recovery efficiency was tested by percentage of spiked analyte (i.e. vitamin D₂) that is recovered when the test sample is analyzed using the entire method. Three different standard concentrations, which are including the matrix of shiitake mushroom, were tested to judge recovery of vitamin D₂ and the analysis was taken in triplicate.

% Recovery was calculated as

$$\% \text{ Recovery} = \frac{X}{Y} \times 100$$

where X = concentration of vitamin D₂ recovered after analysis, Y = concentration of vitamin D₂ standard. 5, 10, and 50 ppm was used and recovery of each concentration was $85.87 \pm 1.97\%$, $92.72 \pm 2.35\%$, and $101.73 \pm 2.05\%$. Linearity value (R^2) of the standard curve was 0.9998.

Validation test and color and texture changes

To verify the validity of the model, the actual vitamin D₂ concentration that was synthesized under optimal conditions was determined in triplicate and compared to the expected vitamin D₂ value predicted by RSM.

To study the color change of UV-irradiated shiitake mushrooms, a HUNTER Lab colorimeter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) was used. The middle layer of the pileus part of five mushroom samples was subjected to color estimation in triplicate after slicing. The measurements were expressed as L^* , a^* , and b^* values, indicating lightness, redness and yellowness, respectively. A color difference (ΔE) between control shiitake mushrooms and UV-irradiated mushrooms was also defined using the following equation:

$$\Delta E = \sqrt{[(L_u^* - L_0^*)^2 + (a_u^* - a_0^*)^2 + (b_u^* - b_0^*)^2]},$$

where subscript 0 refers to the color parameters of the control mushrooms, and L_u^* , a_u^* , and b_u^* are those of UV-irradiated shiitake mushrooms.

A texture analyzer (TAXT plus 50, Stable Micro Systems Ltd., Vienna, UK) was used for texture analysis. Texture evaluations were conducted on 5 mushroom caps. The middle part of the mushroom samples was sliced to a thickness of 1 cm. Samples were compressed with a 5 mm diameter cylinder probe to the same depth. The speed of the probe was 2 mm/s. Hardness, cohesiveness, gumminess, and chewiness were calculated. Both color and texture analyses were performed immediately after vitamin D₂

fortification under optimal conditions, and values of color and texture were compared to those of fresh shiitake mushrooms, set as controls.

Statistical analysis

The RSM procedure and experimental data were analyzed using Statistical Analysis System software (SAS 9.3, SAS Inst. Inc., Cary, N.C., U.S.A.). Statistical analysis of the model was performed using analysis of variance (ANOVA). A student's t test was performed to investigate texture and color differences between UV-treated and untreated mushrooms. A P value of < 0.05 was considered to be significant (SPSS, Statistics 21.0, IBM, U.S.A.).

Results and discussion

Selecting the design variables

We selected three parameters that could affect vitamin D₂ formation in mushrooms. The temperature during UV irradiation is regarded as an important factor that affects vitamin D₂ conversion from ergosterol in mushrooms [10, 13, 17]. The results of the preliminary tests are shown in Fig. 1. Figure 1(A) depicts the conversion of ergosterol to vitamin D₂, which was the highest at 40 °C with 29.47 µg/g (dry mass: DM). Above 40 °C, vitamin D₂ content was reduced. It is expected that increased heat, browning effects, and cell death could lead to a decrease in vitamin D₂ conversion, which was previously reasoned by Jasinghe and Perera [3]. Moreover, it seems that transformation of vitamin D₂ to its by-products such as lumisterol or tachysterol, under elevated temperature conditions [13] also reduced the yield of vitamin D₂.

Figure 1(B) demonstrates the effects of UV dosage on vitamin D₂ yield. Vitamin D₂ from non-irradiated (0 kJ/m²) shiitake mushroom was too low to be detected. Vitamin D₂ yield started to increase after UV irradiation with a UV dosage up to 40 kJ/m² and decreased with a dosage of 50 kJ/m². It is assumed that prolonged exposure to UV radiation might have contributed to an oxidative atmosphere and may result in photo-degradation [10].

Effects of UV wavelength (UV-A, B, and C) on vitamin D₂ production from mushrooms were studied [10, 17], and UV-B ray showed the best conversion efficiency. Simon et al. [18] investigated the effects of UV-B irradiation on compositional changes in white button mushrooms. They reported that there was no significant change in other nutrients, with the exception of vitamin D₂. Therefore, we used a highly concentrated UV-B lamp that radiates a narrow wavelength range (310–311 nm). As higher dosages could lead to decreased quality of mushrooms [6], and

Fig. 1 Effects of independent variables on vitamin D₂ production in shiitake mushrooms. Effects of temperature (A), UV dosage (B), and moisture content (C) on vitamin D₂ synthesis in shiitake mushrooms. Each bar represents mean $\pm$ SD of vitamin D₂ produced and the tests were performed in triplicate. Lower-case letters above bars indicate significant differences between groups ($P < 0.05$)

in consideration of reasonable conditions for industrial processing, we selected the UV dosage range that is listed in Table 1 for the RSM procedure.

The effect of moisture content change on vitamin D₂ yield is shown in Fig. 1(C). Vitamin D₂ content showed the

highest value with a moisture content of 83.5% and decreased above this point. The effect of moisture content on vitamin D₂ conversion was similar to that in previous studies [3, 12]. Moisture content of mushrooms is thought to play an important role in vitamin D₂ [3, 12, 13]. Jasinghe et al. [13] reported that moisture content is considered to be related to the concentration of ergosterol in mushrooms. According to the authors, high moisture content could dilute ergosterol concentration and induce lower conversion efficiency, whereas low moisture levels are thought to be associated with an increase in vitamin D₂ oxidation via exposure to oxygen. Considering that a high deviation in the moisture content of raw mushrooms was observed, ranging from 82 to 88% (data not shown), we decided to designate the highest code level in controllable moisture content range. Based on the results of the preliminary test, the middle points and actual values for the independent variables were coded as shown in Table 1.

Fitting models

The model for vitamin D₂ production with the effects of the three variables was fitted to the following equation:

$$Y = -143.85 - 0.87X_1 + 1.33X_2 + 3.89X_3 - 0.02X_1X_2 + 0.05X_1X_3 + 0.002X_2X_3 - 0.03X_1^2 - 0.01X_2^2 - 0.04X_3^2,$$

where Y denotes expected vitamin D₂ ($\mu\text{g/g DM}$) in shiitake mushrooms, and X_1 , X_2 , and X_3 stand for temperature, UV dosage, and moisture content, respectively. The results for the adequacy and fitness of this model are shown in Table 2. The linear parameters were highly significant ($P < 0.05$), whereas the quadratic and cross parameters were not significant. Therefore, the linear effect of the independent variable was a determining factor in the model. To test the suitability of the regression model, lack of fit testing was also performed. The results indicated that the second-order model was significant at the 95% probability level ($P = 0.0030$) with no significant lack of fit ($P = 0.8999, > 0.05$). The R^2 of the total model for Y was 0.9494. These results indicated that the model for Y was highly suitable to describe the relationships with the independent factors. Although Y was statistically significant for all factors ($P < 0.05$), X_2 was the most significant factor ($P = 0.0028$).

Interpretation and optimization

The response surface plots and contour plots from RSM design are shown in Fig. 2. The combined influence of moisture content and UV dosage, when the temperature was fixed at 35 °C, is depicted in Fig. 2(A). Vitamin D₂ yield

Table 2 ANOVA and lack-of-fit test results for the response surface model

	DF	SS	R ²	F value	P value
Regression					
Linear	3	171.36	0.80	31.34	0.0005
Quadratic	3	8.63	0.04	1.58	0.2897
Cross product	3	25.16	0.12	4.60	0.0534
Total model	9	205.16	0.95	12.51	0.0030
Residual					
Lack of fit	5	6.04	1.21	0.25	0.8999
Pure error	1	4.90	4.90		
Total error	6	10.93	1.82		
Factors	DF	SS	MS	F value	P value
X ₁ —temperature (°C)	4	75.24	18.81	10.32	0.0074
X ₂ —UV dosage (kJ/m ²)	4	109.98	27.50	15.09	0.0028
X ₃ —moisture content (%)	4	51.38	12.84	7.05	0.0188

DF degrees of freedom, SS sum of squares, MS mean squares

increased until the moisture content reached up to around 80% and decreased when the moisture content was raised above this point. Thus, it seems that a moisture content of around 80% is the ideal amount not high enough to dilute the concentration of ergosterol but not too low to increase the surface area of the mushrooms. UV dosage showed positive correlation with vitamin D₂ as vitamin D₂ increased steadily with UV dosage enhancement. Figure 2(B) illustrates the influence of temperature and moisture content, when UV dosage was fixed at 30 kJ/m². Vitamin D₂ yield tended to increase as the temperature decreased when the moisture content was lower up to around 70%. However, temperature caused a linear increase in the response under high levels of moisture. These results imply that a proper temperature range, as well as a suitable moisture content, is needed to obtain maximum vitamin D₂ yield. The effects of temperature and UV dosage on vitamin D₂ yield with a fixed moisture content of 75% are shown in Fig. 2(C). The vitamin D₂ content increased as temperature and UV dosage increased. Increased temperature up to 45 °C, however, induced a decrease in vitamin D₂ content when UV dosage was at high level. It seems that formation of byproducts such as lumisterol or tachysterol, could be one of the factors responsible for a reduction in vitamin D₂ production during irradiation [13]. Thus, UV dosage showed a linear effect toward vitamin D₂ production, whereas temperature and moisture content had a limited range for high vitamin D₂ yield.

The stationary points of each response were saddle points. Therefore, a ridge analysis was performed to determine the critical levels of the design variables that could produce the maximal vitamin D₂ contents. The optimal temperature (X₁), UV dosage (X₂), and moisture

content (X₃) for maximum vitamin D₂ production were 40.56 °C, 36.27 kJ/m², and 80.46%, respectively.

Validation test

The maximum predicted vitamin D₂ value according to the ridge analysis of RSM was 32.33 µg/g (DM). To evaluate the dependability of the experiment model, actual vitamin D₂ contents obtained under the optimal conditions were compared with expected vitamin D₂ contents (Table 3). The actual values of vitamin D₂ produced under the optimal conditions was 29.87 ± 1.38 µg/g (DM) showing that the experimental values are consistent with the maximum predicted value.

Color and texture are important attributes for edible mushrooms to be considered as good products for customers [19, 20]. Therefore, in this study, we compared color and textural changes between fresh and UV irradiated mushroom samples to evaluate the effects of applying the optimal conditions established for the vitamin D₂-fortification process.

Our analysis of color change throughout the vitamin D₂-fortification process is summarized in Table 3. There were no statistically significant changes in lightness (*L** value), redness (*a** value), or yellowness (*b** value) between UV-treated and control mushrooms. The difference in color between UV-treated and untreated shiitake mushrooms was hardly perceptible. The ΔE value was less than 1, which was consistent with a visual assessment. A higher *L** value and a lower ΔE value are considered to be important criteria by the industry for the color quality of dried shiitake mushrooms [20]. As the *L** value showed little difference with untreated mushrooms and ΔE value was less than 1, we confirmed that no adverse effects on the color quality of the area inside of shiitake mushrooms was caused by the vitamin D₂-fortification process.

Textural changes during UV irradiation under optimal conditions are shown in Table 3. There were no significant differences in all textural indices between UV-irradiated and control mushroom samples. The hardness of UV treated mushrooms was slightly increased compared to that of untreated mushrooms. The hardness value represents the force demanded for mastication [20]. It is considered that an increase in hardness is related to removal of moisture and an increase in the concentration of other components in mushrooms [19, 20]. Therefore, it is likely that the vitamin D enhancing process under optimal conditions caused minimal moisture loss and maintained hardness. Food cohesiveness is reported as a factor that could affect working duration in the mouth [21]. Gumminess, which represents energy for disintegration [22], showed no difference after vitamin D₂ fortification nor did cohesiveness. Chewiness, which acts as a function of hardness [19], also

Fig. 2 Response surface plots for optimal vitamin D₂ production. Effects of moisture content and UV dosage on vitamin D₂ content at a constant temperature of 35 °C **(A)**, effects of temperature and moisture on vitamin D₂ content at a constant UV dosage of 30 kJ/m² **(B)**, effects of temperature and UV dosage on vitamin D₂ content at a constant moisture content of 75% **(C)**

scarcely changed after UV irradiation and it seemed to be minimally affected by moisture loss. According to some studies, UV-B irradiation barely changed the proximate

composition of mushrooms including protein, fat, and carbohydrate composition [18, 23]. Therefore, we speculated that no other changes in cell wall-forming nutrients,

Table 3 Predicted and actual vitamin D₂ value and comparison of color, and textural properties under the optimal conditions

Temperature(°C)	Optimal conditions		vit D ₂ content (µg/g)	
	UV dosage (kJ/m ²)	Moisture content (%)	Predicted value	Actual value
40.56	36.27	80.46	32.33	29.87 ± 1.38
Color properties	L*	a*	b*	ΔE
Control	93.18 ± 1	0.47 ± 0.16	8.92 ± 0.71	0.92
UV irradiated	92.4 ± 2.39	0.46 ± 0.48	9.4 ± 1.86	
Textural properties	Hardness (N)	Cohesiveness	Gumminess	Chewiness
Control	11.33 ± 3.24	0.68 ± 0.04	7.65 ± 2.29	6.37 ± 1.89
UV irradiated	12.01 ± 1.82	0.65 ± 0.09	7.67 ± 0.62	6.32 ± 0.76

which are referred as glucans, chitin, and proteins by Zivanovic and others [22], hardly affected texture after the vitamin D₂-fortification process in mushrooms. This study shows our optimal conditions is well matched with the expected value of RSM model and will provide mushroom producers practical and effective information to produce vitamin D₂-fortified shiitake mushrooms, without causing adverse effects on their quality.

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References

- Jasinghe VJ, Perera CO, Barlow PJ. Bioavailability of vitamin D₂ from irradiated mushrooms: an in vivo study. *Brit. J. Nutr.* 93: 951 (2005)
- Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. *Mol. Aspects. Med.* 29: 361–8 (2008)
- Jasinghe VJ, Perera CO. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D₂ by UV irradiation. *Food Chem.* 92: 541–546 (2005)
- Chailurkit LO, Kruavit A, Rajatanavin R. Vitamin D status and bone health in healthy Thai elderly women. *Nutrition* 27: 160–164 (2011)
- Bischoff-Ferrari H. Vitamin D—from essentiality to functionality. *Int. J. Vitam. Nutr. Res.* 82: 321–6 (2012)
- Ko JA, Lee BH, Lee JS, Park HJ. Effect of UV-B exposure on the concentration of vitamin D₂ in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus*). *J. Agr. Food Chem.* 56: 3671–3674 (2008)
- Saif A, Wende K, Lindequist U. In vitro bone inducing effects of *Lentinula edodes* (shiitake) water extract on human osteoblastic cell cultures. *Nat. Prod. Bioprospect.* 3: 282–287 (2013)
- Gu YH, Belury MA. Selective induction of apoptosis in murine skin carcinoma cells (CH72) by an ethanol extract of *Lentinula edodes*. *Cancer lett.* 220: 21–28 (2005)
- Kim MJ, Lee JH, Lee JS. Effect of ultraviolet-B irradiation on antioxidative properties of aqueous extracts from shiitake (*Lentinus edodes*) mushrooms. *Int. J. Food. Sci. Tech.* 49: 2276–2282 (2014)
- Jasinghe VJ, Perera CO. Ultraviolet irradiation: The generator of Vitamin D₂ in edible mushrooms. *Food Chem.* 95: 638–643 (2006)
- Mau J L, Chen PR, Yang JH. Ultraviolet irradiation increased vitamin D-2 content in edible mushrooms. *J. Agr. Food Chem.* 46: 5269–5272 (1998)
- Perera CO, Jasinghe VJ, Ng FL, Mujumdar AS. The Effect of Moisture Content on the Conversion of Ergosterol to Vitamin D in Shiitake Mushrooms. *Dry Technol.* 21: 1091–1099 (2003)
- Jasinghe VJ, Perera CO, Sablani SS. Kinetics of the conversion of ergosterol in edible mushrooms. *J. Food Eng.* 79: 864–869 (2007)
- Baş D, Boyacı İH. Modeling and optimization I: Usability of response surface methodology. *J. Food Eng.* 78: 836–845 (2007)
- Hwang KT, Jung ST, Lee GD, Chinnan MS, Park YS, Park HJ. Controlling molecular weight and degree of deacetylation of chitosan by response surface methodology. *J. Agr. Food Chem.* 50: 1876–1882. (2002)
- Sahin S, Aybastier O, Demir C. Optimization of Ultrasonic-Assisted Extraction of Quercetin and Cyanidin from *Pyracantha Coccinea* and Their Scavenging Effect on Free Radicals. *J. Food Biochem.* 40: 472–479 (2016)
- Wu WJ, Ahn BY. Statistical Optimization of Ultraviolet Irradiate Conditions for Vitamin D-2 Synthesis in Oyster Mushrooms (*Pleurotus ostreatus*) Using Response Surface Methodology. *Plos One* 9 (2014)
- Simon RR, Phillips KM, Horst RL, Munro IC. Vitamin D Mushrooms: Comparison of the Composition of Button Mushrooms (*Agaricus bisporus*) Treated Postharvest with UVB Light or Sunlight. *J. Agr. Food Chem.* 59: 8724–8732 (2011)
- Kotwaliwale N, Bakane P, Verma A. Changes in textural and optical properties of oyster mushroom during hot air drying. *J. Food Eng.* 78: 1207–1211 (2007)
- Wang HC, Zhang M, Mujumdar AS. Comparison of Three New Drying Methods for Drying Characteristics and Quality of Shiitake Mushroom (*Lentinus edodes*). *Dry Technol.* 32: 1791–1802 (2014)
- Nakatsu S, Kohyama K, Watanabe Y, Shibata K, Sakamoto K, Shimoda M. Mechanical properties of softened foodstuffs processed by freeze-thaw infusion of macerating enzyme. *Innov. Food Sci. Emerg.* 16: 267–276 (2012)
- Zivanovic S, Busher R, Kim KS. Textural changes in mushrooms (*Agaricus bisporus*) associated with tissue ultrastructure and composition. *J. Food Sci.* 65: 1404–1408 (2000)
- Zhang Y, Wu WJ, Song GS, Ahn BY. Optimization of Ultraviolet Irradiate Conditions for Vitamin D₂ Synthesis in Shiitake Mushrooms (*Lentinula edodes*) by Using Response Surface Methodology. *J. Appl. Biol. Chem.* 58: 25–29 (2015)