

# Comparison of Ergosterol and Vitamin D<sub>2</sub> in Mushrooms *Agaricus bisporus* and *Cordyceps militaris* Using Ultraviolet Irradiation Directly on Dry Powder or in Ethanol Suspension

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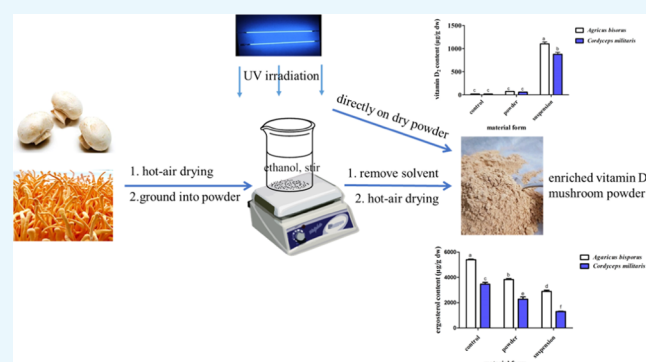
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**ABSTRACT:** Vitamin D deficiency is a severe worldwide health issue. Edible mushrooms are an excellent vitamin D<sub>2</sub> source and have gained popularity worldwide as a nutritional food. The objective of this study was to investigate the conversion efficiency of ergosterol to vitamin D<sub>2</sub> in *Agaricus bisporus* and *Cordyceps militaris* mushrooms under ultraviolet (UV) irradiation directly through dry powder or in ethanol suspension (1:20 g/mL, solid to liquid ratio). Several parameters of UV irradiation conditions such as the material form (dry powder or dry powder in ethanol suspension), exposure time (30, 60, or 120 min), wavelength type (UV-C, UV-B, or UV-A), wavelength combination (UV-C plus UV-B, UV-C plus UV-A, UV-B plus UV-A, or UV-C plus UV-B plus UV-A), and wavelength sequence (UV-C → UV-B, UV-C → UV-A, UV-B → UV-A, or UV-C → UV-B → UV-A), were optimized. Under the optimal UV irradiation conditions (dry powder in ethanol suspension irradiated with UV-C at 40 cm for 120 min), vitamin D<sub>2</sub> concentrations increased from not detectable to 72 μg/g (dw) in the *A. bisporus* dry powder and 1104 μg/g (dw) (about 15-fold increase) in the ethanol suspension. After UV irradiation, the vitamin D<sub>2</sub> concentration increased from undetectable to 57 μg/g (dw) in the *C. militaris* dry powder. In contrast, UV irradiation increased the concentration to 877 μg/g (dw) (about 15-fold higher) in the ethanol suspension. Comparison of the effect of various wavelength combinations showed that UV-C irradiation is more effective than UV-A or UV-B. Furthermore, when irradiated by UV-C at a 40 cm irradiation distance in the ethanol suspension, the increase in vitamin D<sub>2</sub> in *A. bisporus* and *C. militaris* mushrooms was time- or dose-dependent. The conversion rate of vitamin D<sub>2</sub> was low to undetectable under dry powder irradiation, but its ergosterol loss rate was higher than in ethanol suspension irradiation. The ergosterol loss rate in dry *C. militaris* mushrooms was higher than in the dry *A. bisporus* mushroom powder. Ultraviolet irradiation in ethanol suspension could greatly increase the vitamin D<sub>2</sub> concentration than directly on the dry powder and thus make edible mushrooms more practical as a natural vitamin D source for consumers after entirely removing the ethanol.



## 1. INTRODUCTION

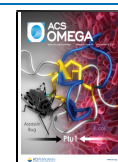
Vitamin D plays an essential role in calcium and phosphorus metabolism and skeletal and neuromuscular homeostasis.<sup>1</sup> Vitamin D deficiency is a major global health issue, particularly in North America, Europe, and the Middle East, where sunlight exposure is limited, especially during the winter. Rickets is a classic symptom of vitamin D deficiency in children, while long-term vitamin D deficiency in adults can eventually cause osteoporosis.<sup>2</sup> In addition, insufficient vitamin D intake often affects the endocrine pancreas and the immune system, leading to various diseases.<sup>3</sup> Vitamin D deficiency is known to be associated with various diseases, such as cancer,<sup>4</sup> heart disease,<sup>5</sup> arterial stiffness,<sup>6</sup> neuropsychiatric disorders,<sup>7</sup> and diabetes.<sup>8</sup> Furthermore, vitamin D has been used as an adjunct to treat COVID-19, which left more than three million people dead as of May 18, 2021.<sup>9</sup>

Mushrooms are a unique source of compounds such as polyphenols, amino acids (i.e., ergothioneine), polysaccharides (i.e., β-glucans), terpenoids, vitamins (i.e., vitamin D<sub>2</sub>), and sterols (i.e., ergosterol). These active components have been linked to antioxidant, anticancer, antidiabetic, anti-inflammatory, hepatoprotective, antiallergic, antimicrobial, and antiviral activities.<sup>10</sup> *Cordyceps militaris* has been extensively studied for its pharmacological activities such as antitumor, anti-inflammation, and immune regulation.<sup>11</sup> Because of their beneficial

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properties, mushrooms have become attractive as functional foods or a source of ingredients that can be extracted and incorporated into food products.

Mushrooms usually contain very little vitamin D<sub>2</sub>, as many are grown in the dark. But mushrooms are notable for being rich in ergosterol (a precursor of vitamin D<sub>2</sub>), which can be transformed to vitamin D<sub>2</sub> by applying natural or artificial ultraviolet (UV) irradiation.<sup>12</sup> UV light consists of three subregions of wavelengths: UV-C (190–290 nm), UV-B (290–320 nm), and UV-A (320–400 nm).<sup>13</sup> The exposure of mushrooms to UV irradiation triggers vitamin D<sub>2</sub> formation. However, the transformation efficiency is affected by many factors, including different types of mushrooms,<sup>14</sup> UV wavelengths,<sup>15</sup> irradiation times,<sup>16</sup> irradiation intensities or doses,<sup>17</sup> irradiation parts,<sup>18</sup> and moisture contents.<sup>19</sup> Previous research has focused on the fresh or dried mushrooms directly treated with single-wavelength UV-A, UV-B, or UV-C irradiation.<sup>17,20</sup> For example, the concentration of vitamin D<sub>2</sub> in shiitake mushrooms was increased after exposure to UV-B at a dose of 25 kJ/m<sup>2</sup>. As the exposure area increased, the vitamin D<sub>2</sub> concentration was increased more effectively by exposure slices than exposure to the gill or pileus of the whole mushrooms.<sup>17a</sup> UV-C irradiation provides an efficient way to increase the vitamin D<sub>2</sub> concentration in mushrooms.<sup>20</sup> Improving the nutritional value of mushrooms with UV irradiation, making it a facile available source of vitamin D, deserves further investigation.

Fresh mushrooms deteriorate rapidly after harvest because of the high water content. Therefore, drying is typically used to extend its shelf life. Therefore, exposing dried edible fungi to UV irradiation to increase the vitamin D<sub>2</sub> content can further increase the nutritional value of edible fungi and improve the current situation of vitamin D deficiency. However, most existing literature focused on the content changes of the vitamin D<sub>2</sub> single compound. However, in the process of ultraviolet irradiation of dried edible fungi, the changes in ergosterol and vitamin D<sub>2</sub> content in edible fungi under various irradiation conditions are of great significance for the development and application of foods and drugs to enhance ergosterol and vitamin D<sub>2</sub>. Still, there are a few studies on ultraviolet irradiation to improve vitamin D<sub>2</sub> of *C. militaris*.

Hence, this study focused on the ergosterol and vitamin D<sub>2</sub> content treated with UV irradiation in *Agaricus bisporus* and *C. militaris* mushroom powders directly or in ethanol suspension with different irradiation distances and times, different single wavelengths, various combinations of wavelengths, and different sequences of combined wavelengths.

## 2. MATERIALS AND METHODS

**2.1. Materials.** Fresh *A. bisporus* (Strain W-192) and *C. militaris* (Strain JN-168) mushrooms were purchased from a local supermarket supplied by Yinong Co. (Mianxian, Shaanxi, China, 33.15° N and 106.67° E) and Meiao Co. (Dongying, Shandong, China, 38.15° N and 118.50° E), respectively. The analytical-grade anhydrous alcohol and the chromatographic-grade formic acid and acetonitrile were purchased from Aladdin Co. (Shanghai, China). The standard vitamin D<sub>2</sub> and ergosterol were provided by Aldrich Co. (GR, Shanghai, China).

**2.2. Sample Preparation.** The preparation of the mushroom powder was carried out by reference methods<sup>21</sup>.

**2.3. UV Irradiation.** **2.3.1. Exposure with Different Irradiation Material Forms.** The mushroom dry powder

(4.0 g) and anhydrous alcohol (80 mL) were mixed in a 250 mL glass beaker with a material–liquid ratio of 1:20 (g/mL). The mixtures were exposed to UV-C irradiation in a UV chamber (the length, width, and height are 40, 50, and 50 cm, respectively) with nine UV-C lamps (40 cm, Philips, TUV PL-L 36W/4p) at a distance of 40 cm from the UV lamps for 2 h with magnetic stirring at 500 rpm/min (C-MAG HS7 digital magnetic stirrer, IKA Co., Staufen, Germany). The irradiation intensity was 2.74 mW/cm<sup>2</sup>, and the calculated exposure dose was 197.28 kJ/m<sup>2</sup>. Ethanol was then entirely removed at 40 °C under 0.1 MPa by a rotary evaporator (RV 10, IKA Co., Staufen, Germany). The mushroom powder was further dried<sup>21</sup> and named as the suspension sample. Correspondingly, identical quantities of *A. bisporus* and *C. militaris* mushroom powders were directly treated with UV-C radiation for 2 h by interval stirring using a stainless steel fork every 10 min in a beaker. The obtained mushroom solid was named as the powder sample. Conversely, the *A. bisporus* or *C. militaris* mushroom powder free of any irradiation was used as a control sample.<sup>21,22</sup>

**2.3.2. Exposure to UV-C with Different Irradiation Times and Distances.** The mixtures (mushroom dry powder or dry powder in ethanol suspension) were irradiated by UV-C at a distance of 30 or 40 cm from UV lamps in a UV chamber (the length, width, and height are 40, 50, and 50 cm, respectively) with nine UV-C lamps (40 cm, Philips, TUV PL-L 36W/4p) for 0, 30, 60, or 120 min with magnetic stirring at 500 rpm/min. The exposure intensities were 3.29 and 2.74 mW/cm<sup>2</sup> at distances of 30 and 40 cm, respectively. The sample processing method used was the same as described in Section 2.3.1.<sup>21</sup>

**2.3.3. Irradiation with Different Single Wavelengths.** The mixtures (mushroom dry powder or dry powder in ethanol suspension) were irradiated with UV-A, or UV-B, or UV-C at a distance of 10 cm from the UV lamps in a UV chamber (the length, width, and height are 30, 27, and 16 cm, respectively) for 2 h. The exposure intensities were 4.81, 1.67, or 1.11 mW/cm<sup>2</sup>, respectively. The sample processing method used was the same as described in Section 2.3.1.<sup>21</sup>

**2.3.4. Irradiation with Different Combinations of Wavelengths.** The mixtures (mushroom dry powder or dry powder in ethanol suspension) were exposed to UV-C plus UV-B, UV-C plus UV-A, UV-B plus UV-A, or UV-C plus UV-B plus UV-A radiation at a distance of 10 cm from the UV lamps in a UV chamber (the length, width, and height are 30, 27, and 16 cm, respectively) for 2 h. The sample processing method is the same as described in Section 2.3.1.<sup>21</sup>

**2.3.5. Irradiation with Different Sequences of Combined Wavelengths.** The mixtures (mushroom dry powder or dry powder in ethanol suspension) were exposed to different sequences of combined wavelengths, i.e., UV-C (2 h) → UV-B (2 h), UV-C (2 h) → UV-A (2 h), UV-B (2 h) → UV-A (2 h), or UV-C (2 h) → UV-B (2 h) → UV-A (2 h) at a distance of 10 cm from the UV lamps in a UV chamber (the length, width, and height are 30, 27, and 16 cm, respectively). The sample processing method is the same as described in Section 2.3.1.<sup>21</sup>

**2.4. Sample Preparation.** Ergosterol and vitamin D<sub>2</sub> were extracted without separation following our previous method.<sup>21</sup>

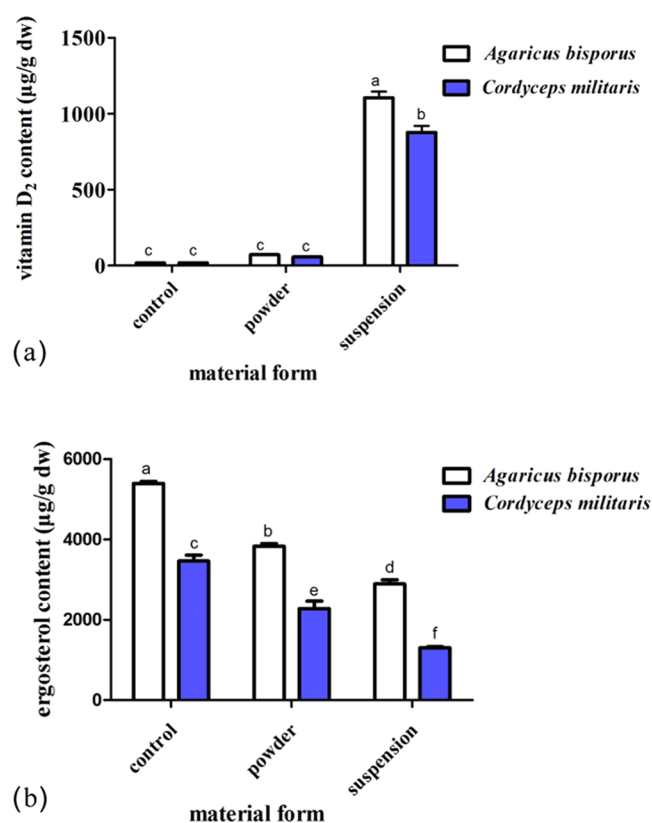
**2.5. Qualitative and Quantitative Determination of Ergosterol and Vitamin D<sub>2</sub>.** Qualitative and quantitative determination of ergosterol and vitamin D<sub>2</sub> was conducted by HPLC analysis following our reported method.<sup>21</sup>

**2.6. Statistical Analysis.** Analysis of variance (ANOVA) was used to analyze the data. Significant differences between

treatments were determined using Duncan's HSD. SPSS 16.0 for Windows was used to conduct all statistical procedures. A significance level of  $p < 0.05$  was selected to separate the mean for all analyses.

### 3. RESULTS AND DISCUSSION

#### 3.1. Influence of Different Material Forms on the Vitamin D<sub>2</sub> and Ergosterol Concentrations in Mushrooms *A. Bisporus* and *C. Militaris* Exposed to UV-C Irradiation with Different Material Forms. Figure 1 shows



**Figure 1.** Influence of different material forms on the vitamin D<sub>2</sub> (a) and ergosterol (b) concentrations in mushrooms *A. bisporus* and *C. militaris* exposed to UV irradiation with different material forms. Different letters indicate significant differences ( $p < 0.05$ ).

the influence on vitamin D<sub>2</sub> and ergosterol concentrations in mushrooms *A. bisporus* and *C. militaris* exposed to UV-C irradiation with different material forms. Material forms significantly affect the vitamin D<sub>2</sub> content. In both mushrooms, *A. bisporus* and *C. militaris*, the content of vitamin D<sub>2</sub> irradiated upon dry powder was remarkably lower than that irradiated in ethanol suspension.

The results indicated that the content of vitamin D<sub>2</sub> in mushrooms *A. bisporus* and *C. militaris* could significantly increase by exposure to UV-C in an alcohol suspension. The vitamin D<sub>2</sub> content was increased by 6152 and 4845% in mushrooms *A. bisporus* and *C. militaris*, respectively ( $p < 0.05$ ) (Figure 1a). Nevertheless, the ergosterol content was significantly lost in *A. bisporus* and *C. militaris* after 2 h of treatment, which was about 46 and 62%, respectively (Figure 1b). Therefore, it can be speculated that ergosterol may be transformed into other photoisomerization products such as tachysterol and lumisterol under UV treatment in a dry powder state. However, the structures and concentrations of photo-

isomerization products need to be further verified in future studies.

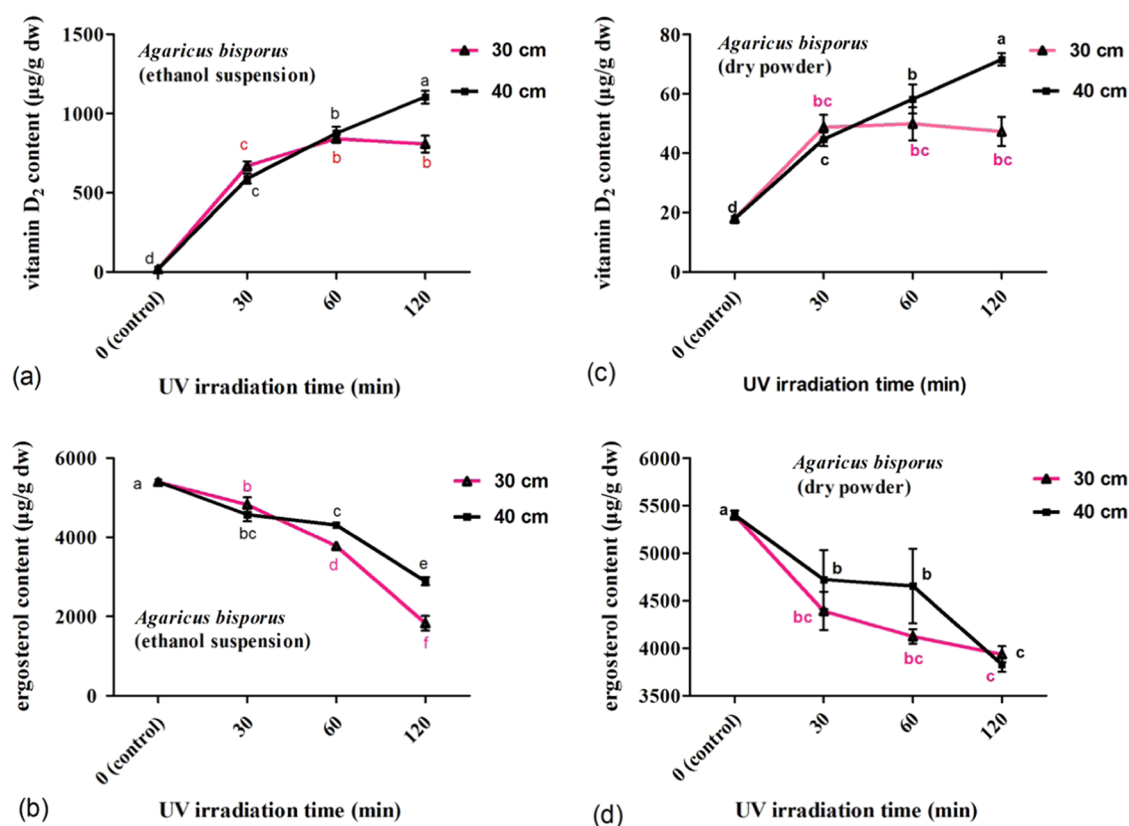
For the mushrooms *A. bisporus*, the vitamin D<sub>2</sub> content increased ( $p < 0.05$ ) with an increased rate of 36 to 72 µg/g for the dry powder form. However, the vitamin D<sub>2</sub> content significantly increased ( $p < 0.05$ ) from not detectable to 1104 µg/g with an increased rate (a value divided by the total duration of the experiment) of 552 µg/h for the ethanol suspension. Therefore, the content of vitamin D<sub>2</sub> exposure in ethanol suspension is about 15 times that of dry powder (Figure 1a). In contrast with the control, the content of ergosterol decreased ( $p < 0.05$ ) to only 29 and 46% for the dry powder and the ethanol suspension, respectively (Figure 1b).

For the mushrooms *C. militaris*, the vitamin D<sub>2</sub> content increased ( $p > 0.05$ ) with an increased rate of 28–57 µg/g for the dry powder form. However, the vitamin D<sub>2</sub> content significantly increased ( $p < 0.05$ ) from not detectable to 877 µg/g with an increased rate of 438 µg/h for the ethanol suspension (Figure 1a). In contrast with the control, the content of ergosterol decreased ( $p < 0.05$ ) to only 34 and 62% for the dry powder and the ethanol suspension, respectively (Figure 1b).

It has been reported that 70–80% was the optimum moisture content of mushrooms when exposed to UV irradiation.<sup>23</sup> Vitamin D<sub>2</sub> could be easily oxidized and photodegraded as the exposure to an oxygen and oxidative atmosphere increased.<sup>24</sup> These research studies indicate that mushrooms with UV irradiation in the ethanol suspension were more effective in increasing vitamin D<sub>2</sub> concentration compared with direct exposure of the dry powder. This result can be interpreted as follows: when irradiated in ethanol suspension with continuous stirring, the exposure area and UV penetration increased, and the oxidation and photodegradation of vitamin D<sub>2</sub> could be avoided.

**3.2. Influence of Different Material Forms on the Vitamin D<sub>2</sub> and Ergosterol Concentrations in Mushrooms *A. Bisporus* and *C. Militaris* Exposed to UV-C Irradiation at Different Times and Distances.** Irradiation with UV-C could increase ( $p < 0.05$ ) the vitamin D<sub>2</sub> content in a time or dose-dependent manner for mushrooms *A. bisporus* and *C. militaris*. But the increase rates were decreased with prolonged irradiation time. Correspondingly, the preferable irradiation distance was 40 cm (Figures 2a–d and 3a–d). UV-C irradiation decreased ( $p < 0.05$ ) the ergosterol content in mushrooms *A. bisporus* and *C. militaris*. The content of ergosterol in mushrooms *A. bisporus* was less impacted than that in mushrooms *C. militaris*.

For mushroom *A. bisporus* irradiated in ethanol suspension at 30 cm from the UV lamps, the content of vitamin D<sub>2</sub> increased gradually ( $p < 0.05$ ) from not detectable to 670 and 808 µg/g for 30 and 120 min irradiation, respectively. The increase rates decreased ( $p < 0.05$ ) steadily from 1340 to 404 µg/h (Figure 2c). The content of ergosterol decreased ( $p < 0.05$ ) steadily from 89 and 34% for 30 and 120 min irradiation, respectively (Figure 2d). When irradiated in ethanol suspension at a 40 cm distance, the vitamin D<sub>2</sub> content in mushrooms *A. bisporus* increased ( $p < 0.05$ ) from not detectable to 591 and 1104 µg/g for 30 and 120 min irradiation, respectively. The increase rates decreased ( $p < 0.05$ ) gradually from 1182 to 552 µg/h (Figure 2c). Correspondingly, the content of ergosterol decreased ( $p < 0.05$ ) steadily from 85 and 54% for 30 and 120 min irradiation, respectively (Figure 2d).



**Figure 2.** Influence of UV irradiation on the vitamin D<sub>2</sub> and ergosterol concentrations in mushrooms *A. bisporus* exposed to UV irradiation at different times and distances. The concentrations of vitamin D<sub>2</sub> (a) and ergosterol (b) in *A. bisporus* mushroom irradiated in ethanol suspension by various treatments. The concentrations of vitamin D<sub>2</sub> (c) and ergosterol (d) in *A. bisporus* mushroom irradiated upon dry powder by various treatments.

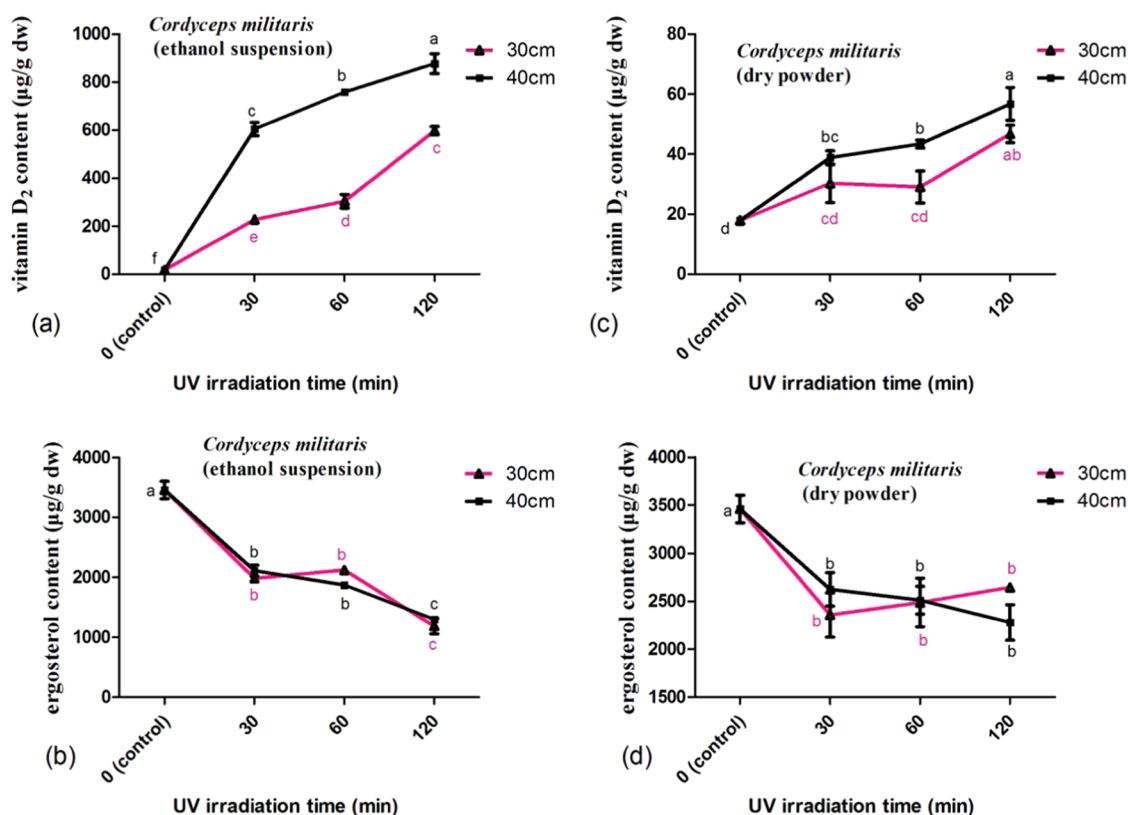
For mushroom *C. militaris* irradiated in ethanol suspension 30 cm from the UV lamps, the vitamin D<sub>2</sub> content increased ( $p < 0.05$ ) gradually from not detectable to 304 and 597  $\mu\text{g/g}$  for 30 and 120 min irradiation, respectively. The increase rates decreased ( $p < 0.05$ ) steadily from 608 to 299  $\mu\text{g/h}$  (Figure 3c).

Correspondingly, the content of ergosterol decreased ( $p < 0.05$ ) steadily from 57 and 34% for 30 and 120 min irradiation, respectively (Figure 3d). When irradiated in ethanol suspension at a 40 cm distance, the vitamin D<sub>2</sub> content in mushrooms *C. militaris* increased ( $p < 0.05$ ) from not detectable to 604 and 877  $\mu\text{g/g}$  for 30 and 120 min irradiation, respectively. The increase rates decreased ( $p < 0.05$ ) gradually from 1208 to 439  $\mu\text{g/h}$  (Figure 3c). Correspondingly, the content of ergosterol decreased ( $p < 0.05$ ) steadily from 61 and 38% for 30 and 120 min irradiation, respectively (Figure 3d).

Irradiation with UV-C tended to decrease the content of ergosterol for mushrooms *A. bisporus* and *C. militaris*. More ergosterol content decreased with the prolonged irradiation time. In addition, this trend in mushrooms *C. militaris* was more evident than that in mushrooms *A. bisporus*. With the extension of the irradiation time, the ergosterol content of *A. bisporus* in ethanol suspension decreased significantly ( $p < 0.05$ ). The difference was significant among different irradiation distances at the same time. The ergosterol content in *C. militaris* gradually decreased with an increase in the irradiation time ( $p > 0.05$ ), and there was no significant difference between different irradiation distances at the same

time. For example, with UV-C exposure at 40 cm for 120 min, the content of ergosterol in mushrooms *A. bisporus* decreased ( $p < 0.05$ ) to 71 and 54% upon dry powder and in ethanol suspension, respectively. In contrast, the content of ergosterol in mushrooms *C. militaris* decreased ( $p < 0.05$ ) to 66 and 38% (Figures 2b,d and 3b,d). Previous studies showed that for fresh white and brown button mushrooms, the content of ergosterol decreased insignificantly for the UV-C treatment of 50, 100, and 200 s.<sup>25</sup> UV-C irradiation of fresh white button mushrooms resulted in no significant reduction in the ergosterol content.<sup>26</sup>

The content of ergosterol in edible mushrooms differed from various strains after UV exposure.<sup>13</sup> With the prolonged UV-B or UV-C irradiation time, the content of ergosterol remarkably decreased for mushrooms *A. bisporus* but inversely increased for mushrooms *A. bitorquis* and *Volvariella volvacea*.<sup>13</sup> For example, after UV-B or UV-C irradiation, the content of ergosterol in mushrooms *A. bitorquis*, *V. volvacea*, and *L. edodes* increased to 502% for 2 h, 126% for 1 h, and 125% for 1 h compared with untreated samples.<sup>13</sup> After UV-B irradiation for dry powder, the content of ergosterol increased in button mushrooms but decreased in all other mushroom types.<sup>14</sup> This study indicated that the content of ergosterol in mushrooms *C. militaris* lowered ( $p < 0.05$ ) from 3461  $\mu\text{g/g}$  of unexposure to 1984  $\mu\text{g/g}$  for 30 min of irradiation in ethanol suspension and then increased ( $p > 0.05$ ) to 2122  $\mu\text{g/g}$  for 60 min (Figure 3d). Further research is needed to understand the change of ergosterol as a result of UV-C irradiation.



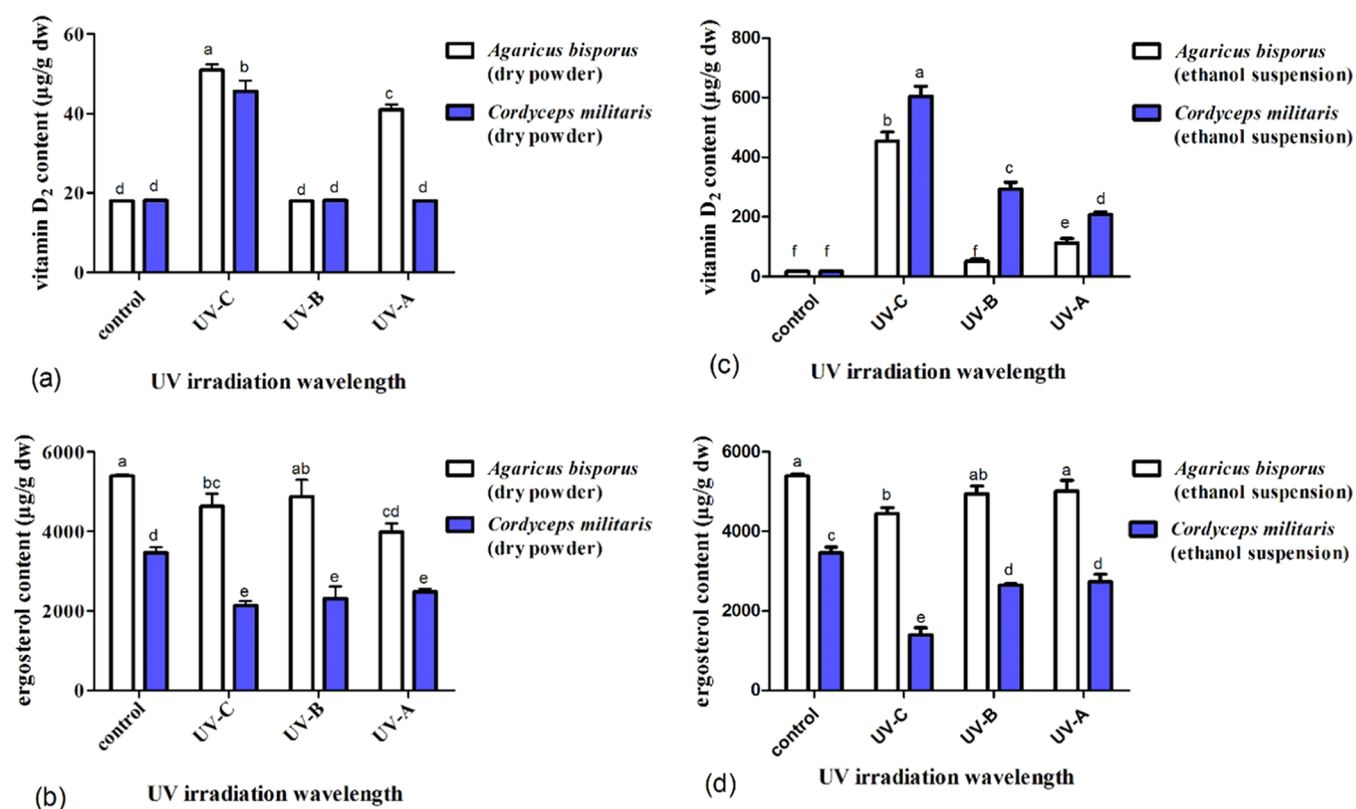
**Figure 3.** Influence of UV irradiation at different times and distances on the vitamin D<sub>2</sub> and ergosterol concentrations in mushrooms *C. militaris*. The concentrations of vitamin D<sub>2</sub> (a) and ergosterol (b) in the *C. militaris* mushroom irradiated in ethanol suspension by various treatments. The concentrations of vitamin D<sub>2</sub> (c) and ergosterol (d) in the *C. militaris* mushroom irradiated upon dry powder by various treatments.

For both mushrooms *A. bisporus* and *C. militaris*, UV-C exposure could promote vitamin D<sub>2</sub> transformation, but the increased rates in mushrooms *C. militaris* were lower than in mushrooms *A. bisporus*. Nevertheless, the increased rates in both *A. bisporus* and *C. militaris* mushrooms are far beyond reported by Mau et al.<sup>13</sup> This discrepancy most likely is due to the higher irradiation intensity and the suspension of ethanol.

**3.3. Effect of UV Irradiation at Different Single Wavelengths on the Vitamin D<sub>2</sub> and Ergosterol Concentrations in Mushrooms *A. Bisporus* and *C. Militaris*.** Various irradiation wavelengths greatly impact the content of vitamin D<sub>2</sub>, and the influences vary with different mushrooms. For the mushrooms *A. bisporus*, both UV-C and UV-A exposures are more efficient than UV-B exposure. The vitamin D<sub>2</sub> content increased about 2.8 times and 2.3 times upon dry powder and 8.8 times and 2.2 times in ethanol suspension compared to UV-B. There is a significant difference between UV-A and UV-C (Figure 4a,c). When irradiated upon dry powder, the content of vitamin D<sub>2</sub> increased from not detectable for the untreated control to 51 μg/g for UV-C ( $p < 0.05$ ), followed by 41 μg/g for UV-A ( $p < 0.05$ ) and not detectable for UV-B ( $p > 0.05$ ), with the increase rates gradually decreased from 26 μg/h for UV-C to 21 μg/h for UV-A ( $p < 0.05$ ) (Figure 4a). When irradiated in ethanol suspension, the vitamin D<sub>2</sub> content increased from not detectable for the untreated control to 454 μg/g for UV-C ( $p < 0.05$ ), followed by 113 μg/g for UV-A ( $p < 0.05$ ) and 52 μg/g for UV-B ( $p > 0.05$ ), with the increase rates gradually decreased from 227 μg/h for UV-C to 57 μg/h for UV-A ( $p < 0.05$ ) (Figure 4c). Correspondingly, the conversion rate of vitamin D<sub>2</sub> was low to undetectable under dry powder

irradiation, but its ergosterol loss rate was higher than that in ethanol suspension irradiation. For example, the concentration of ergosterol steadily decreased ( $p < 0.05$ ) to 90% for UV-B, 74% for UV-A, and 86% for UV-C when irradiated upon dry powder (Figure 4b). When irradiated in ethanol suspension, the concentration of ergosterol steadily decreased ( $p < 0.05$ ) to 92% for UV-B, 93% for UV-A, and 82% for UV-C (Figure 4d). When irradiated upon dry powder for UV-B, 10% ergosterol was lost even though vitamin D<sub>2</sub> was undetectable (Figure 4a,b).

For the *C. militaris* mushrooms, UV-C irradiation is more effective than UV-B and UV-A irradiation. In dry powder, the content of vitamin D<sub>2</sub> is 46 μg/g for UV-C compared to undetectable for both UV-B and UV-A. The content of vitamin D<sub>2</sub> is approximately 2.1 and 2.9-fold increased in ethanol suspension compared to UV-B and UV-A, and there is a significant difference between UV-B and UV-A (Figure 4a,c). When irradiated in ethanol suspension, the vitamin D<sub>2</sub> content increased from not detectable for the untreated control to 605 μg/g for UV-C ( $p < 0.05$ ), followed by 294 μg/g for UV-B ( $p < 0.05$ ) and the minimum value of 208 μg/g for UV-A ( $p > 0.05$ ), with rates gradually decreased ( $p < 0.05$ ) from 303 μg/h for UV-C, 147 μg/h for UV-B, and 104 μg/h for UV-A (Figure 4c). In contrast, the conversion rate of vitamin D<sub>2</sub> was low to undetectable under dry powder irradiation, but its ergosterol loss rate was higher than that in ethanol suspension irradiation. For example, the ergosterol content sharply decreased to 40% for UV-C ( $p < 0.05$ ), 76% for UV-B ( $p > 0.05$ ), and 79% for UV-A ( $p < 0.05$ ) (Figure 4d). When irradiated upon dry powder, the concentration of ergosterol steadily decreased ( $p < 0.05$ ) to 62% for UV-C, 67% for UV-B, and 72% for UV-A



**Figure 4.** Influence of UV irradiation at different single wavelengths on the vitamin D<sub>2</sub> and ergosterol concentrations in mushrooms *A. bisporus* and *C. militaris*. The concentrations of vitamin D<sub>2</sub> (a) and ergosterol (b) in *A. bisporus* and *C. militaris* mushrooms irradiated upon dry powder by various treatments. The contents of vitamin D<sub>2</sub> (c) and ergosterol (d) in *A. bisporus* and *C. militaris* mushrooms irradiated in ethanol suspension by various treatments.

(Figure 4b). When irradiated upon dry powder for UV-B and UV-A, 33 and 29% ergosterol were lost, respectively, even though vitamin D<sub>2</sub> was undetectable (Figure 4a,b).

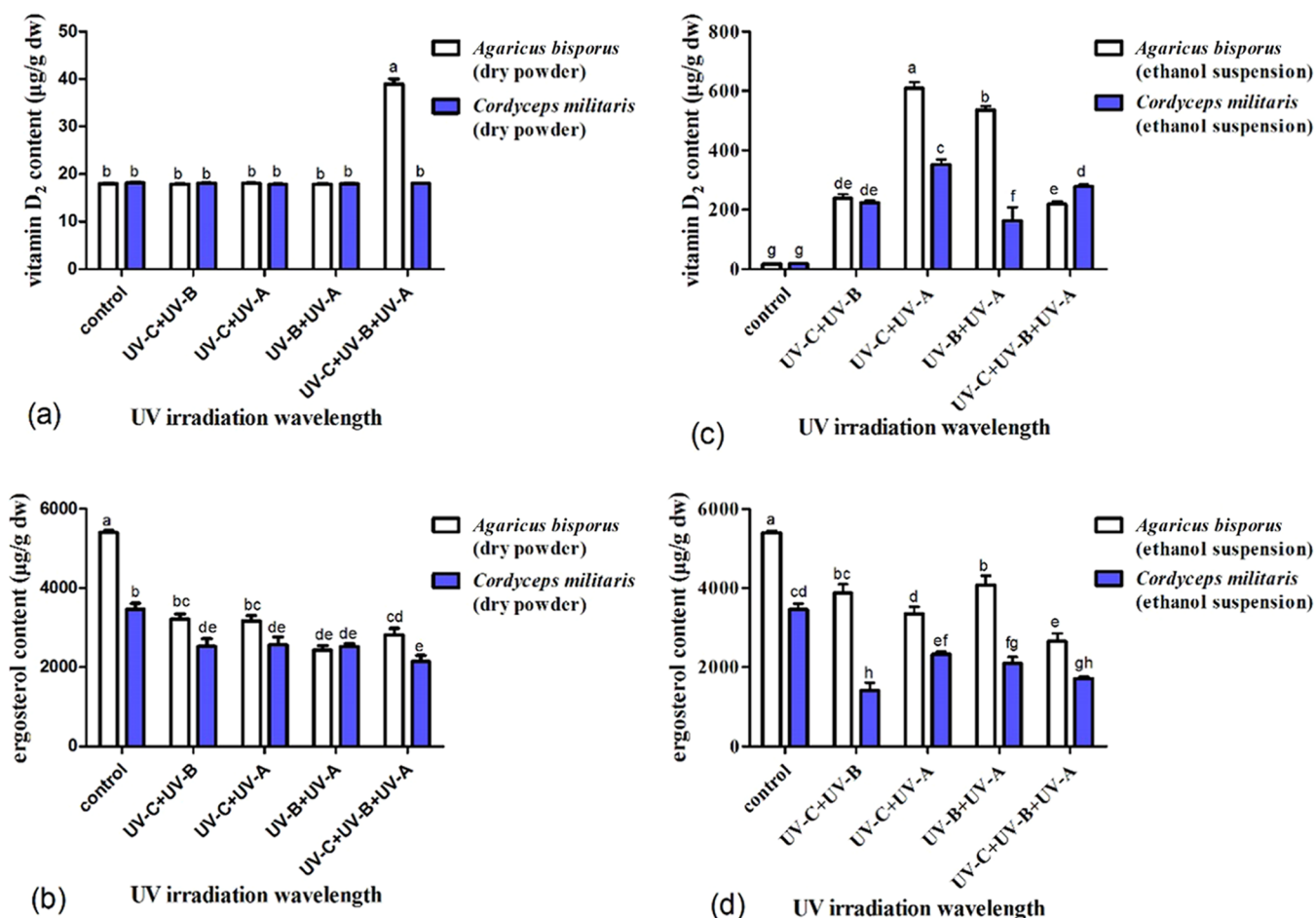
UV irradiation could remarkably increase the content of vitamin D<sub>2</sub> in edible mushrooms, and an essential factor is the UV irradiation wavelength. In addition, tachysterol is more likely to be produced by light treatment with a shorter wavelength, while the content of lumisterol is increased with a longer wavelength.<sup>14</sup>

Exposing the gills of mushrooms to UV-A irradiation for 2 h, one could obtain high levels of vitamin D<sub>2</sub>. Among the four edible mushrooms tested, the maximum vitamin D<sub>2</sub> conversion was obtained for oyster mushrooms. Conversely, the minimum conversion was obtained for button mushrooms under a 2 h exposure in a calculated dosage of 25.2 kJ/m<sup>2,23</sup>

When exposed to UV-B irradiation, the vitamin D<sub>2</sub> content increased for fresh and dried mushrooms.<sup>22,27</sup> In addition, dried mushrooms can produce ergocalciferol under UV-B irradiation.<sup>27</sup> The content of vitamin D<sub>2</sub> in lyophilized mushrooms *A. bisporus* was 42–119 µg/g (dw), and that in hot-air dried mushrooms was 22–81 µg/g (dw).<sup>27</sup> Compared with untreated samples, the vitamin D<sub>2</sub> content in the caps and stems increased for both brown and white button mushrooms exposed to UV-C irradiation.<sup>25</sup>

**3.4. Influence of UV Irradiation at Different Combination Wavelengths on the Vitamin D<sub>2</sub> and Ergosterol Concentrations in Mushrooms *A. Bisporus* and *C. Militaris*.** Various combinations of irradiation wavelengths significantly impact the content of vitamin D<sub>2</sub>, and the influences vary with different mushrooms. For the *A. bisporus*

mushrooms, the content of ergosterol was sharply decreased ( $p < 0.05$ ) even though vitamin D<sub>2</sub> was undetectable when irradiated upon dry powder for UV-C plus UV-B, UV-C plus UV-A, and UV-B plus UV-A. When irradiated upon dry powder, the vitamin D<sub>2</sub> concentration only increased ( $p < 0.05$ ) to 39 µg/g for UV-C plus UV-B plus UV-A, but was still undetectable for UV-C plus UV-B, UV-C plus UV-A, and UV-B plus UV-A (Figure 5a). But the concentration of ergosterol sharply decreased ( $p < 0.05$ ) to 45% for UV-B plus UV-A, 52% for UV-C plus UV-B plus UV-A, 58% for UV-C plus UV-A, and 60% for UV-C plus UV-B (Figure 5b). When irradiated in ethanol suspension, the vitamin D<sub>2</sub> concentration increased ( $p < 0.05$ ) from not detectable to a maximum value of 609 µg/g for UV-C plus UV-A, followed by 536 µg/g for UV-B plus UV-A, 239 µg/g for UV-C plus UV-B, and a minimum value of 219 µg/g for UV-C plus UV-B plus UV-A, with increase rates steadily decreasing from 305 to 268 ( $p < 0.05$ ), 120 ( $p < 0.05$ ), and 110 µg/h ( $p < 0.05$ ). The difference between UV-C plus UV-A, UV-B plus UV-A, or UV-C plus UV-B was significant, but there was a significant difference between UV-C plus UV-B and UV-C plus UV-B plus UV-A (Figure 5c). Correspondingly, the concentration of ergosterol gradually decreased ( $p < 0.05$ ) to 76% for UV-B plus UV-A, 72% for UV-C plus UV-B, 62% for UV-C plus UV-A, and 49% for UV-C plus UV-B plus UV-A. The difference in the ergosterol concentration between UV-C plus UV-B plus UV-A and UV-C plus UV-B was insignificant. But there was a significant difference between UV-C plus UV-A, UV-B plus UV-A to UV-C plus UV-B plus UV-A or UV-C plus UV-B (Figure 5d).



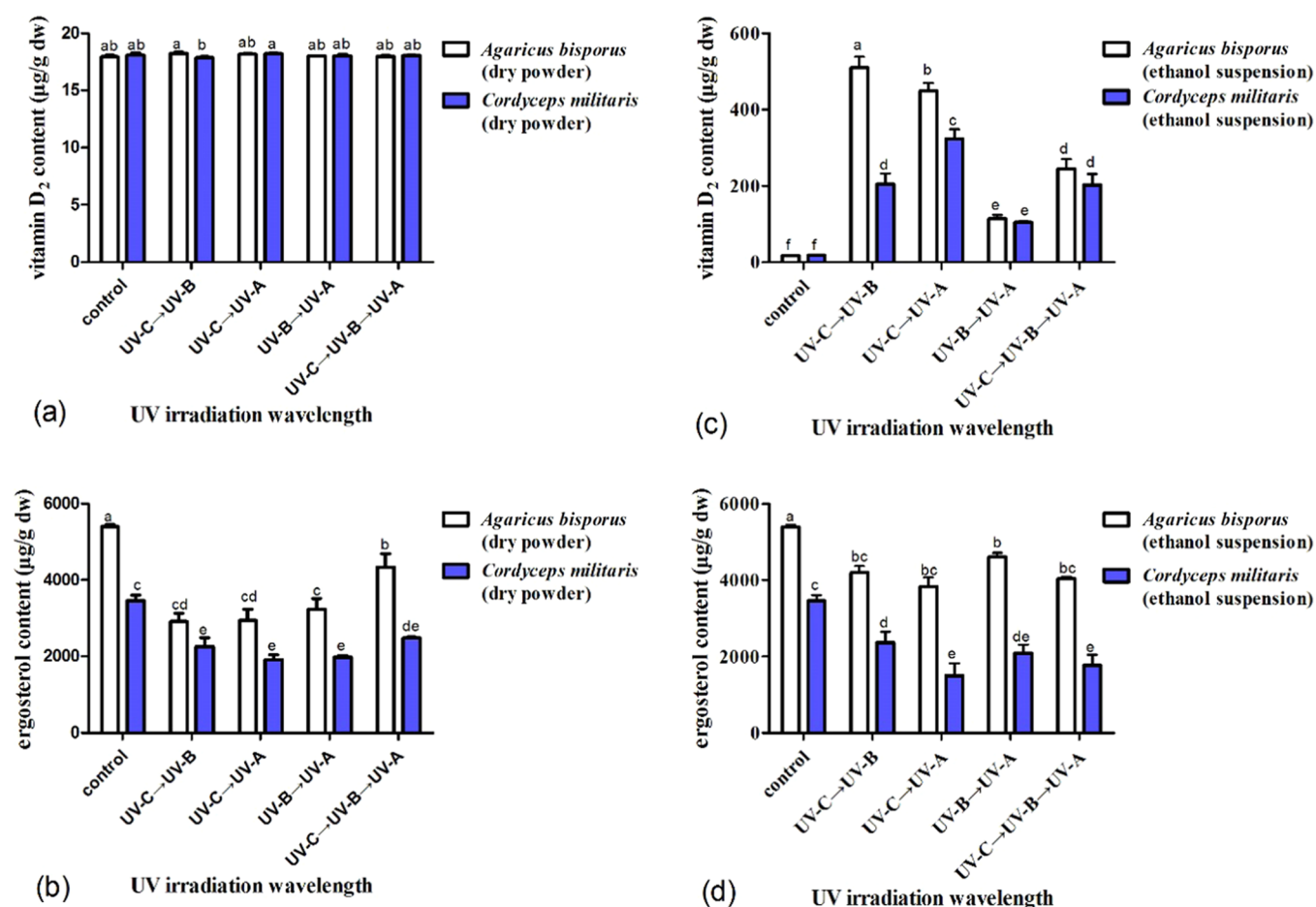
**Figure 5.** Influence of UV irradiation at different combinations of wavelengths on the vitamin D<sub>2</sub> and ergosterol concentrations in mushrooms *A. bisporus* and *C. militaris*. The concentrations of vitamin D<sub>2</sub> (a) and ergosterol (b) in *A. bisporus* and *C. militaris* mushrooms irradiated upon dry powder by various treatments. The contents of vitamin D<sub>2</sub> (c) and ergosterol (d) in *A. bisporus* and *C. militaris* mushrooms irradiated in ethanol suspension by various treatments.

For the *C. militaris* mushrooms, when irradiated upon dry powder, the vitamin D<sub>2</sub> content is still not detectable for four treatments (Figure 5a), but the content of ergosterol gradually decreased ( $p < 0.05$ ) to 74% for UV-C plus UV-A, 73% for UV-C plus UV-B and UV-B plus UV-A, and 62% for UV-C plus UV-B plus UV-A (Figure 5b). On the other hand, when irradiated in ethanol suspension, the vitamin D<sub>2</sub> content increased ( $p < 0.05$ ) from not detectable to a maximum value of 352 µg/g for UV-C plus UV-A, followed by 278 µg/g for UV-C plus UV-B plus UV-A, 223 µg/g for UV-C plus UV-B, and a minimum value of 163 µg/g for UV-B plus UV-A, with increase rates steadily decreasing from 176 to 139 ( $p < 0.05$ ), 112 ( $p < 0.05$ ), and 82 µg/h ( $p < 0.05$ ). Therefore, the difference between UV-C plus UV-A, UV-B plus UV-A, UV-C plus UV-B plus UV-A or UV-C plus UV-B was significant. But, there was no significant difference between UV-C plus UV-B and UV-C plus UV-B plus UV-A, similar to the mushrooms *A. bisporus* (Figure 5c). Correspondingly, the content of ergosterol gradually decreased ( $p < 0.05$ ) to 67% for UV-C plus UV-A, 61% for UV-B plus UV-A, 50% for UV-C plus UV-B plus UV-A, and 41% for UV-C plus UV-B (Figure 5d).

**3.5. Influence of UV Irradiation at Different Sequences of Combined Wavelengths on the Vitamin D<sub>2</sub> and Ergosterol Concentrations in Mushrooms *A. Bisporus* and *C. Militaris*.** Various sequences of combined wavelengths greatly affect the vitamin D<sub>2</sub> content, which is different with

varied mushrooms. For the *A. bisporus* mushroom, when irradiated upon dry powder, the vitamin D<sub>2</sub> content is still not detectable for four treatments (Figure 6a). But the concentration of ergosterol gradually decreased ( $p < 0.05$ ) to 80% for UV-C → UV-B → UV-A, 60% for UV-B → UV-A, and 54% for UV-C → UV-B and UV-C → UV-A (Figure 6b). When irradiated in ethanol suspension, the vitamin D<sub>2</sub> concentration increased ( $p < 0.05$ ) from not detectable for the untreated control to a maximum of 510 µg/g for UV-C → UV-B, followed by 449 µg/g for UV-C → UV-A, and 245 µg/g for UV-C → UV-B → UV-A, and a minimum of 114 µg/g for UV-B → UV-A, with the rate of increase decreased gradually from 128 to 112 ( $p < 0.05$ ), 41 ( $p < 0.05$ ), and 29 µg/h ( $p < 0.05$ ). The difference between these four treatments was significant (Figure 6c). Correspondingly, the concentration of ergosterol decreased steadily to 85% for UV-B → UV-A ( $p < 0.05$ ), 78% for UV-C → UV-B ( $p < 0.05$ ), 75% for UV-C → UV-B → UV-A ( $p < 0.05$ ), and 71% for UV-C → UV-A ( $p < 0.05$ ) (Figure 6d). This result is consistent with the sequential wavelength irradiation of a *Lentinus edode* ethanol suspension, but the ergosterol loss rate was higher than that of *L. edodes* treatment.

For the *C. militaris* mushrooms, when irradiated upon dry powder, the vitamin D<sub>2</sub> concentration is still not detectable for the four treatments (Figure 6a), but the ergosterol concentration gradually decreased ( $p < 0.05$ ) to 72% for



**Figure 6.** Influence of UV irradiation at different sequences of combined wavelengths on the vitamin D<sub>2</sub> and ergosterol concentrations in mushrooms *A. bisporus* and *C. militaris*. The concentrations of vitamin D<sub>2</sub> (a) and ergosterol (b) in *A. bisporus* and *C. militaris* mushrooms irradiated upon dry powder by various treatments. The contents of vitamin D<sub>2</sub> (c) and ergosterol (d) in *A. bisporus* and *C. militaris* mushrooms irradiated in ethanol suspension by various treatments.

UV-C → UV-B → UV-A, 65% for UV-C → UV-B, 57% for UV-B → UV-A, and 55% for UV-C → UV-A (Figure 6b). When irradiated in ethanol suspension, the vitamin D<sub>2</sub> concentration increased ( $p < 0.05$ ) from not detectable for the untreated control to 322 µg/g for UV-C → UV-A, followed by 205 µg/g for UV-C → UV-B, 203 µg/g for UV-C → UV-B → UV-A, and 105 µg/g for UV-B → UV-A, with the rate of increase gradually decreased ( $p > 0.05$ ) from 81 to 51, 33, and 26 µg/h. (Figure 6c). This tendency was consistent with the results of sequential wavelength irradiation of *Pleurotus ostreatus* in ethanol suspension, but the content of vitamin D<sub>2</sub> varies with the variety of edible fungi. The concentration of ergosterol steadily decreased to 76% for UV-C → UV-B, 60% for UV-B → UV-A ( $p < 0.05$ ), 51% for UV-C → UV-B → UV-A ( $p > 0.05$ ), and 43% for UV-C → UV-A ( $p > 0.05$ ). But, the difference between UV-C → UV-A, UV-B → UV-A, and UV-C → UV-B → UV-A was insignificant. The ergosterol loss rate of *C. militaris* irradiated in ethanol suspension was higher than in *A. bisporus*, shiitake mushrooms, and oyster mushrooms (Figure 6d). For *A. bisporus* and *C. militaris* mushrooms, concentrations of ergosterol and vitamin D<sub>2</sub> varied with the different sequences of combined wavelength treatments.

After UV irradiation, provitamin D could absorb energy and undergo several reverse photoreactions. The referred isomers mainly include ergosterol, provitamin D<sub>2</sub>, vitamin D<sub>2</sub> tachysterol, and lumisterol. This study focused on the effects

of a different single wavelength and combined wavelength treatments on the content of ergosterol and vitamin D<sub>2</sub>. The transformation mechanism between different isomers remains to be further investigated,

#### 4. CONCLUSIONS

The results obtained from the present study demonstrated that the irradiated approach could enormously increase the vitamin D<sub>2</sub> content. For both *A. bisporus* and *C. militaris* mushrooms, samples exposed to UV irradiation in ethanol suspension could greatly increase the vitamin D<sub>2</sub> content than that directly upon dry powder. The vitamin D<sub>2</sub> content increased with the increase in the UV irradiation exposure time and dosage. However, the prolonging of exposure time resulted in the reduction of the increase rate. Moreover, the irradiation distance of 40 cm was preferable. UV-A or UV-C treatment or a combination of both were more effective than UV-B irradiation. Under the optimal UV irradiation conditions (dry powder in ethanol suspension with UV-C exposure at 40 cm for 120 min), the concentrations of vitamin D<sub>2</sub> increased from not detectable to 1104 µg/g (dw) and 877 µg/g (dw) in *A. bisporus* and *C. militaris* mushroom powders in the ethanol suspension, respectively. In addition, the ergosterol content was decreased with the increase in vitamin D<sub>2</sub> content, and the ergosterol loss rate in dry *C. militaris* mushrooms was higher than that in dry *A. bisporus* mushrooms. Thus, exposure to UV



irradiation in ethanol suspension efficiently increases the vitamin D<sub>2</sub> content, and consequently, enhances the nutritional value of edible mushrooms, increases natural sources of vitamin D supplementation, and is beneficial to consumer health after thoroughly removing the solvent ethanol.

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