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Sterol and vitamin D_2 contents in some wild and cultivated mushrooms

Pirjo Mattila[a,*], Anna-Maija Lampi[b], Riitta Ronkainen[b], Jari Toivo[b], Vieno Piironen[b]

[a]*Food Research, Agricultural Research Centre of Finland, Building L, FIN-31600 Jokioinen, Finland*
[b]*Department of Applied Chemistry and Microbiology, University of Helsinki, PO Box 27 (Latokartanonkaari 11), FIN-00014 University of Helsinki, Finland*

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Abstract

The contents of vitamin D_2 and sterols in some wild and cultivated mushrooms were determined, and the distribution of these compounds in different parts of the wild mushrooms was evaluated. In addition, the variation in vitamin D_2 contents between individual fruiting bodies of wild mushrooms was studied. Vitamin D_2 was determined using an HPLC method, including saponification and semipreparative normal-phase HPLC purification before analytical reversed-phase quantification with an internal standard. Sterol contents were analysed with gas chromatography using an internal standard method, including saponification before derivatizing sterols to trimethylsilyl ethers. Mass-spectral analyses were used to further confirm the identification of sterols. Vitamin D_2 was almost totally absent in cultivated mushrooms, while some wild mushrooms contained high concentrations of this vitamin (4.7–194 μg/100 g dry weight). Ergosterol was the most abundant sterol found in mushrooms, and its contents were higher in cultivated mushrooms (602.1–678.6) than in wild mushrooms (296–489 mg/100 g dry weight). The contents of vitamin D_2 and ergosterol varied greatly and moderately, respectively, between different parts of the mushrooms and were lowest in stipes. In addition, high variation in vitamin D_2 contents between individual fruiting bodies was found.

Keywords: Vitamin D_2; Sterols; Ergosterol; Mushrooms; Fungi

1. Introduction

The origin of ergosterol and vitamin D_2 (ergocalciferol) lies in the kingdom Fungi. Vitamin D_2 is derived by photoirradiation from its precursor ergosterol. Ergosterol undergoes photolysis when exposed to UV light of wavelengths 280–320 nm to yield a variety of photoirradiation products, the principal ones being previtamin D_2, tachysterol and lumisterol. The previtamin D_2 undergoes spontaneous thermal rearrangement to vitamin D_2 (Jones, Seamark, Trafford, & Makin, 1985; Parrish, 1979; Singh, 1985). In the kingdoms Plantae and Animalia, ergosterol or vitamin D_2 are almost absent. However, low levels of these compounds can be found in plants if they are contaminated with mould or yeast (Young & Games, 1993). Analytical methods to determine ergosterol in foods are based on either those detecting such contaminants in cereals and other foods or those examining the taxonomy or phylogenesis of fungi (Staffas, 2001; Weete & Gandhi, 1997; Young, 1995).

Vitamin D is an essential compound for humans. It can be produced in the skin by the action of sunlight or absorbed from the diet in the intestinal tract. The intake of vitamin D from food is especially emphasized in the northern (or southern) latitudes. Furthermore, some population groups, e.g. vegetarians, are at special risk of receiving insufficient levels of this vitamin from their diet (Lamberg-Allardt, Kärkkäinen, Seppänen, & Biström, 1993). Although vitamin D deficiency is classically associated with rickets and osteomalacia, milder deficiency states can, lead to osteoporosis (Eastell & Riggs, 1997).

In previous studies, we found that wild mushrooms are a rich natural vitamin D source (Mattila, Piironen, Uusi-Rauva, & Koivistoinen, 1994), which is also

* Corresponding author. Tel.: +358-3-4188-3235; fax: +358-3-4188-3266.
E-mail address: pirjo.mattila@mtt.fi (P. Mattila).

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accepted by most vegetarians. In addition, at least from freeze-dried *Chantarellus tubaeformis,* the bioavailability of vitamin D_2 was favourable (Outila, Mattila, Piironen, & Lamberg-Allardt, 1999). However, Mattila et al. (1994) studied only pooled samples, and more specific information on the distribution of vitamin D_2 in different parts of the mushrooms and variation in individual mushrooms is needed.

More advanced fungi produce ergosterol as the principle sterol, differing from major plant sterols in having two double bonds in the sterol ring structure instead of one. Fungal and plant sterols are biosynthetized in similar reactions, but the sequence of postsqualene reactions and the stereochemistry of the major products differ. Most fungal sterols, e.g. ergosterol, contain 28 carbon atoms (Parks & Weete, 1991). Various minor sterols, present in fungi, have been identified, including fungisterol, ergosta-5,7-dienol, 24-methyl cholesterol and methylene cholesterol (Lyznik & Wojciechowski, 1981; Weete & Gandhi, 1997). The fungal sterols provide characteristic functions that are necessary for vegetative growth. Fungi can, however, accept different sterols in their membranes, but then they must adjust the membrane structure by modifying the phospholipid composition.

Plant sterols are recognized as having positive health effects, because they have been shown to decrease serum cholesterol levels and may also be beneficial in preventing colon cancer, as reviewed by Piironen, Lindsay, Miettinen, Toivo, and Lampi (2000). Fungal sterols may have similar functions to other plant sterols, there are however, few data on their nutritional effects.

The aim of the present study was to evaluate the vitamin D_2 and sterol contents of wild and cultivated mushrooms and to learn more of their distribution in different parts of mushrooms and between individuals. Finally, the vitamin D_2 and ergosterol contents in these edible mushrooms were compared, since vitamin D_2 is biosynthetized from ergosterol.

2. Materials and methods

2.1. Sampling

The sterol and vitamin D contents were determined from cultivated *Pleurotus ostreatus, Agaricus bisporus*/brown, *A. bisporus*/white and *Lentinus edodes* in summer 2000. These mushrooms were obtained from the major mushroom producers in Finland. The mushrooms (1.5 kg of each species) were cut into 1-cm^3 cubes, mixed, packed into 300-ml plastic containers with 50–70 g in each, freeze-dried and stored at −18 °C. Before sterol analysis, the contents of the container were homogenized. The sample handling for vitamin D_2 analysis and results of vitamin D contents are presented elsewhere (Mattila et al., 2001).

In the case of wild mushrooms, the mean vitamin D and sterol contents in each species, as well as the distribution of sterols and vitamin D_2 in different parts of the mushrooms (pilei, stipes, gills) and variation in vitamin D_2 contents in individual mushrooms, were studied. Wild mushrooms, were purchased in summer 1998 from a marketplace in Helsinki (*Chantarellus cibarius*; 10 l) or from mushroom pickers (*C. tubaeformis*, *Boletus edulis* and *Lactarius trivialis;* 3 kg of each). Five litres of *C. cibarius* and 1.5 kg of other wild mushrooms were cut into pieces, mixed and pooled according to species for determination of the mean vitamin D and sterol contents. Four mushrooms, of approximately equal weight of each species, were chosen to study variation in individual mushrooms. They were stored separately at −18 °C until analysis, and homogenized immediately before determination. From the rest of the mushrooms the pilei, stipes, and gills were separated, pooled according to species, stored at −18 °C until analysis and homogenized immediately before analysis. In addition, all the mushroom samples were analysed for dry matter.

2.2. Vitamin D

The vitamin D_2 (ergocalciferol) contents of the wild mushroom samples were determined using previously validated methods of Mattila, Piironen, Bäckman, Asunmaa, Uusi-Rauva, and Koivistoinen (1992) and Mattila et al. (1994). The fresh mushroom sample (2–10 g) was saponified and extracted with a mixture of petroleum and diethyl ether and, after evaporation of the extract, the residue was dissolved in 2 ml of *n*-hexane. A 500-μl aliquot of the *n*-hexane extract was purified using isocratic normal-phase semipreparative HPLC. After semipreparative purification the collected fraction was evaporated and dissolved in 150 μl of 7% water in methanol. An amount of 100 μl was then injected into the analytical reversed-phase HPLC system. Quantification was based on the use of an internal standard method employing vitamin D_3. The analysis of vitamin D_2 and its contents in cultivated mushrooms is described elsewhere (Mattila et al. 2001).

2.3. Sterols

The sterol contents of wild and cultivated mushroom samples were determined by gas chromatography, using an internal standard method. Homogenized freeze-dried samples (0.1 g) mixed with cholesterol as an internal standard (160–400 μg; 99 + %, Sigma Chemical Co. St Louis, MO, USA), were saponified with 0.5 ml of saturated aqueous KOH in 8 ml of absolute ethanol at 85 °C for 30 min. The unsaponifiable matter was extracted, with 20 ml of cyclohexane, from the hydrolysate diluted with 12 ml of water. One tenth of the extract was dried

under a gentle nitrogen stream at 50 °C. The sterols were derivatized to trimethylsilyl (TMS) ethers and analysed by gas chromatography using flame ionization detection, as described previously by Toivo, Lampi, Aalto, and Piironen (2000), except that an RTX-5w/Integra fused silica capillary column (60 m×0.32 mm i.d., cross-bonded 5% diphenyl-95% dimethyl polysiloxane, 0.1 μm film; Restek Corp.) was used with constant helium flow of 0.7 ml/min. All samples were analysed in triplicate.

The sterols identified and analysed in cultivated mushrooms were ergosterol (5,7,22-cholestatrien-24β-methyl-3β-ol), ergosta-7,22-dienol (7,22-cholestadien-24β-methyl-3β-ol), ergosta-5,7-dienol (5,7-cholestadien-24β-methyl-3β-ol) and fungisterol (7-cholesten-24β-methyl-3β-ol). Ergosterol was quantitatively analysed in wild mushrooms, while the other sterols were semi-quantitatively measured.

Quantitation of all sterols was based on the calibration performed for ergosterol (5,7,22-cholestatrien-24β-methyl-3β-ol; Steraloids Inc). The concentrations of the ergosterol solutions used were checked by UV spectroscopy ($\lambda = 282$ nm; $\epsilon = 11\,900\ \mathrm{l \times mol^{-1}\ cm^{-1}}$; in absolute ethanol; $M_w = 396.6$ g/mol). To calculate a linear calibration curve, mixtures of 5–200 μg of ergosterol and 20 μg of cholesterol were analysed in duplicate.

Comparison with literature data (Rahier & Benveniste, 1989) and mass spectral analyses were used to further confirm the identification of ergosterol and identify the other sterols. A gas chromatograph, equipped with a mass spectrometer (Varian 3400 GC; FinniganMat Incos 50 MS), was used to produce the mass spectra. The sterols were separated in the same column as described above at a constant temperature of 270 °C. For mass spectroscopy, ionization energy of 70 eV in the electron impact mode was used and spectra were scanned in the range of m/z 100–600. Ion-source and transfer-line temperatures were 160 and 270 °C, respectively.

Ergosterol, ergosta-7,22-dienol, ergosta-5,7-dienol and fungisterol TMS ethers showed molecular ions (M^+) of m/z 468, 470, 470 and 472, respectively. Ergosterol and ergosta-5,7-dienol showed a major fraction of m/z 143 that is characteristic for sterols with two double bonds in the ring structure. Their base peaks were m/z 363 and 365 that represent M^+ without a TMS and with an additional methyl group. Ergosta-7,22-dienol and fungisterol had a significant peak at m/z 255 that is typical for Δ7 sterols and ergosta-7,22-dienol at m/z 342, suggesting a double bond in the side chain at C-22 (Lyznik & Wojciechowski, 1981; Rahier & Benveniste, 1989).

Recovery of ergosterol was studied using a mixed in-house standard sample of freeze-dried cultivated *A. bisporus*. The sample (0.1 g) was spiked with 0.39 and 0.78 mg of ergosterol. The recovery of added ergosterol was 94.6% ($n = 6$).

3. Results and discussion

3.1. General

Wild mushrooms, *C. cibarius, C. tubaeformis, B. edulis* and *Lactarius trivialis,* were chosen for subjects of investigation because all these mushrooms are popular in Finland, and they differ in their crop season and/or their morphology. In addition, more information on the vitamin D_2 and ergosterol contents of the *Chantarellus* species was desired, because previous data suggested that they contain high levels of vitamin D_2 (Mattila et al., 1994). We have previously reported the vitamin D_2 contents of the major mushrooms cultivated in Finland: *A. bisporus*/brown, *A. bisporus*/white, *P. ostreatus*, and *Lentinus edodes* (Mattila et al., 2001). In the present study the sterol contents of these mushrooms are presented (Table 1).

3.2. Vitamin D_2 and sterol contents in whole mushrooms

Mattila et al. (2001) showed that vitamin D_2 was almost totally absent in cultivated mushrooms *A. bisporus*/brown, *A. bisporus*/white, *P. ostreatus*, and *Lentinus edodes* ($\leqslant$ 0.1 μg/100 g fresh weight). On the other hand, wild mushrooms (especially *C. tubaeformis* and *C. cibarius*) contained high concentrations of this vitamin (Table 2). The main reason for this is that cultivated mushrooms are grown in the dark in Finland and, as mentioned in the introduction, sunlight and UV irradiation affect the vitamin D_2 contents in mushrooms, converting ergosterol to ergocalciferol. Mattila et al. (1994) also showed that wild mushrooms contained much higher amounts of vitamin D_2 (2.91–29.82 μg/100 g fresh weight) than dark-cultivated *A. bisporus* (0.21 μg/100 g fresh weight); similarly the highest contents were found in *C. tubaeformis*. On the other hand, if grown under natural climatic conditions, cultivated *Lentinus edodes* could also contain high amounts of vitamin D_2, ranging from 22 to 110 μg/100 g dry weight (Takamura, Hoshino, Sugahara, & Amano, 1991).

Cultivated mushrooms contained high amounts of sterols (Table 1). The range of total content of analysed sterols was from 625 to 774 mg/100 g dry weight. Only closely related C_{28} sterols were detected, namely ergosterol, ergosta-7,22-dienol, ergosta-5,7-dienol and fungisterol. Ergosterol was the most abundant sterol in all cultivated mushrooms studied, comprising 83–89% of the total sterol content. All the sterols detected have also been found earlier in higher fungi, and ergosterol has been found to be the major sterol constituent in most mushrooms (Huang, Yung, & Chang, 1985; Lyznik & Wojciechowski, 1981; Parks & Weete, 1991; Senatore, 1992; Yokokawa & Mitsuhashi, 1981). Somewhat higher ergosterol contents were reported for cultivated mushrooms in the present study than earlier by

Table 1
Sterol contents in cultivated mushrooms (mg/100 g dry weight)

	Ergosterol	Ergosta-7,22-dienol	Ergosta-7,5-dienol	Fungisterol	Dry matter (%)
Agaricus bisporus/white	654	15.2	94.0	25.8	7.7
A bisporus/brown	602	14.6	47.1	13.5	7.8
Pleurotus ostreatus	674	15.4	83.3	17.6	8.0
Lentinus edodes	679	16.7	28.5	62.7	8.4

Table 2
Vitamin D_2 (μg/100 g dry weight) and sterol contents (mg/100 g dry weight) in different parts of the wild mushrooms and vitamin D_2 contents in individual mushrooms

	Vitamin D_2	Ergosterol	Dry matter (%)
Chantarellus cibarius[a]			
Whole	84	304	11.1
Pileus	102	140	9.9
Stipe	24	100	10.4
Gill	115	278	13.1
Mushroom 1	24		9.3
Mushroom 2	129		10.0
Mushroom 3	150		9.7
Mushroom 4	55		11.8
Chantarellus tubaeformis			
Whole	194	377	7.0
Pileus	337	367	5.7
Stipe	187	345	7.6
Gill	104	439	9.3
Mushroom 1 (9.3 g[b])	196		6.3
Mushroom 2 (9.3 g)	169		6.9
Mushroom 3 (9.2 g)	410		7.4
Mushroom 4 (11.8 g)	324		5.9
Boletus edulis			
Whole	4.7	489	12.4
Pileus	22	589	10.4
Stipe	2.1	444	12.2
Gill	2.0	549	12.2
Mushroom 1 (121 g)	9.8		11.1
Mushroom 2 (89.5 g)	4.8		10.7
Mushroom 3 (99.7 g)	25		11.2
Mushroom 4 (85.6 g)	5.7		13.7
Lactarius trivialis			
Whole	29	296	6.0
Pileus	53	335	4.9
Stipe	7.3	235	7.0
Gill	6.0	346	8.0
Mushroom 1 (63.2 g)	24		5.9
Mushroom 2 (50.8 g)	30		6.2
Mushroom 3 (45.5 g)	39		6.9
Mushroom 4 (54.8 g)	11		6.4

[a] Information of weights of individual mushrooms is missing
[b] Weight of individual mushrooms

Huang et al. (1985) and Koyama, Aoyagi, and Sugahara (1984). Ergosterol contents can, however, vary greatly in mushrooms, e.g. Trigos, Martinez-Carrera, Hernandez, and Sobal (1997) found that the variation in ergosterol contents in fruiting bodies of *Pleurotus* is high, depending on strains, species and external factors (light, substrate and the presence of sodium acetate). In addition, Huang et al. (1985) found that ergosterol contents varied highly in different *Lentinus edodes* strains.

The second principal sterol in cultivated *A. bisporus*/white, *A. bisporus*/brown, and *P. ostreatus* was ergosta-5,7-dienol, whose percentage varied from 7% to 12% while, in *Lentinus edodes,* the second principal sterol was fungisterol, comprising 8% of the total sterol content (Table 1). Scant information is available on sterols other than ergosterol in the mushrooms studied. Yokokawa and Mitsuhashi (1981) found no fungisterol at all in *Lentinus edodes*; only ergosterol and ergosta-5,7-dienol were found. On the other hand, Huang et al. (1985) found fungisterol and ergosta-5,7-dienol in addition to ergosterol. The contents of the last two sterols were similar. Lyznik and Wojciechowski (1981) detected similar sterols, in *Agaricus campestris,* to those analysed in this study for *Agaricus* varieties and *P. ostreatus*. They showed that ergosterol and ergosta-5,7-dienol comprised 78.7 and 13.6%, respectively, of the total sterol content of 32.6 mg/kg fresh weight.

The ergosterol contents in the wild mushrooms *C. cibarius, C. tubaeformis*, *B. edulis,* and *Lactarius trivialis* ranged from 296 to 489 mg/100 g dry weight and were thus similar to those reported in Huang et al. (1985) and Koyama et al. (1984). In addition to ergosterol, *C. cibarius* contained ergosta-5,7-dienol, *Lactarius trivialis* ergosta-7,22-dienol and fungisterol, and *B. edulis* all three minor C_{28} sterols mentioned above. The amounts of these other sterols were approximately 10, 30 and 15% of the total sterol contents, respectively. Ergosterol was the only sterol identified in *C. tubaeformis*.

The vitamin D_2 contents of whole mushrooms did not follow the contents of its precursor ergosterol, since the range of vitamin D_2 content was much broader (1–194 μg/100 g dry weight) than that of ergosterol (296–679 mg/100 g dry weight). Cultivated mushrooms were better sources of ergosterol than wild mushrooms.

3.3. Vitamin D_2 and sterol contents in different parts of the wild mushrooms

The effect of light was seen in the vitamin D_2 results for the different mushroom parts, i.e. pileus, stipe and gills. In *C. tubaeformis, B. edulis* and *Lactarius lactarius,* the

pileus, which is most affected by sunlight, contained the highest amounts of vitamin D_2. In *C. cibarius,* the gills also contained high amounts of vitamin D_2, while the concentration of this vitamin in the stipe was clearly lower. This was probably caused by the morphology and mode of growth of *C. cibarius*, which enable more exposure of sunlight on the gills than in the other mushrooms studied. To our knowledge there is no previous information concerning the distribution of vitamin D_2 in different parts of mushroom fruiting bodies. On the other hand, other nutrients have been found to exist in different concentrations in the pileus and stipe. For example, Poongkodi and Sakthisekaran (1995) showed that the contents of proteins, carbohydrates, lipids, ascorbic acid, tocopherols and many minerals were higher in the pilei of cultivated *Pleurotus* and *Agaricus* than in their stipes.

The ergosterol contents also varied in different parts of the wild mushrooms, but not so strongly as vitamin D_2. The ergosterol (like vitamin D_2) contents were clearly lowest in stipes. Huang et al. (1985) analysed the ergosterol content of the pileus and stipe of *Volvariella volvacea* and found the same phenomenon. Since the stipe supports more weight than the pileus, it would be expected that it would show a greater percentage of structural polysaccharides. The increase in these structural polysaccharides therefore accounts for a lower percentage of other cell constituents, e.g. sterols (Huang et al., 1985). In the case of *Lentinus edodes*, however, the lowest ergosterol contents were found in the pileus and highest in the gills (Ono et al., 1974). As shown in Table 2, the gills were also the richest ergosterol sources of *C. cibarius*, *C. tubaeformis* and *Lactarius trivialis* investigated in the present study.

3.4. Variation in vitamin D_2 contents between individual fruiting bodies

The variation in vitamin D_2 contents between individual fruiting bodies of the same species was significant and appeared to be independent of the weight of the mushroom (Table 2). In the present study, it was not possible to gather information on the habitat of the individual fruiting bodies but referring to the text above, it is very probable that the fruiting bodies, containing lower amounts of vitamin D_2, were grown in the dark. To our knowledge there are no previously published data concerning the variation in vitamin D_2 contents in individual fruiting bodies. However, it is typical of vitamin D that variation in its contents occurs in foods; good examples for this are fishes and eggs (Mattila et al, 1992, 1995; Mattila, Piironen, Haapala, Hirvi, & Uusi-Rauva, 1997; Mattila, Piironen, Hakkarainen, Hirvi, Uusi-Rauva, & Eskelinen, 1999). This large variation should be accounted for when compiling nutrient databases and giving recommendations for daily allowances.

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