Vitamin D Contents in Edible Mushrooms†

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The ergocalciferol and 25-hydroxyergocalciferol contents in cultivated *Agaricus bisporus* and in five different wild mushroom species were determined by high-performance liquid chromatography (HPLC), using internal standard methods, and the level of previtamin D_2 was screened. The methods included saponification and extraction, purification using one or two semipreparative HPLC steps, and quantification with analytical HPLC. The contents of ergocalciferol found in different mushroom species varied significantly (0.21–29.82 μg/100 g of fresh weight). Wild mushrooms, especially *Cantharellus cibarius* and *Cantharellus tubaeformis*, contained high amounts of ergocalciferol, 12.8 and 29.82 μg/100 g of fresh weight, respectively. The contents of 25-hydroxyergocalciferol were below the limit of detection in all mushrooms. The contribution of previtamin D_2 to the total vitamin D activity was less than 10% of that of ergocalciferol in the mushroom species studied.

Keywords: *Vitamin D; ergocalciferol; vitamin D_2; 25-hydroxyergocalciferol; previtamin D_2; edible mushrooms; HPLC; food*

INTRODUCTION

The compositions of edible wild mushrooms have not been much studied, possibly because wild mushrooms are not considered to be a significant part of the diet. However, interest in wild mushrooms and in possibilities of deriving benefit from the abundance of mushroom harvests in the forests has been raised in Finland. The cultivation of mushrooms has also increased in Finland in recent years.

To our knowledge there are no up-to-date data on the vitamin D contents in wild mushrooms. In the 1930s the vitamin D contents in edible mushrooms (e.g., *Cantharellus cibarius, Boletus edulis, Psalliota* sp., and *Gyromitra esculenta*) were studied by Scheunert et al. (1935). They found quite notable contents in those species, 63–125 IU/100 g of fresh weight (equivalent to 1.6–3.1 μg of ergocalciferol/100 g). Food composition tables seldom include vitamin D contents of wild mushrooms. Among the food composition tables most frequently used in Europe (Holland et al., 1991; Möller, 1985; Souci et al., 1986), only the German table (Souci et al., 1986) gives figures for some species. These values are, however, mainly based on the work of Scheunert et al. (1935).

Slightly more knowledge of the vitamin D contents in cultivated mushrooms (e.g., *Agaricus bisporus* and *Lentinus edodes)* is available but varies significantly with and within different species. A content of only 0–2 μg/100 g of fresh weight has been found in *A. bisporus* (Souci et al., 1986; Holland et al., 1991; Möller, 1985) but from 0.04 (Takeuchi et al., 1984) to 21.8–109.6 μg/100 g of dry matter (Takamura et al., 1991) in *L. edodes*.

The aim of this study was to produce up-to-date data on the contents of vitamin D-active compounds in wild mushrooms and cultivated *A. bisporus*.

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MATERIALS AND METHODS

Standards. The ergocalciferol and cholecalciferol standards were obtained from Sigma Chemical Co., and the 25-hydroxycholecalciferol standard was from Duphar B. V., Vitamins and Chemicals Division, Holland. The 25-hydroxyergocalciferol standard was a generous gift from F. Hoffman-LaRoche Ltd., Switzerland. The stock and working standard solutions of the standards were made as described earlier (Mattila et al., 1992, 1993).

The previtamin D_2 and previtamin D_3 were prepared by heating the ergocalciferol and cholecalciferol standards (about 10 μg in 2 mL of methanol) in a boiling-water bath for 1 h. The previtamins were separated from the vitamins using straight-phase high-performance liquid chromatography (HPLC), and the identity and retention times of the previtamins were confirmed using reversed-phase HPLC with a diode array detector (see Procedures).

Sampling. All samples of mushrooms were gathered in the late summer–early autumn of 1993. One-liter samples of *C. cibarius* and *Cantharellus tubaeformis* were bought from six different retailers in two local markets in Helsinki. The same amounts of each subsample of *C. cibarius* and *C. tubaeformis* (200 and 150 g, respectively) were weighed, pooled, lyophilized, and vacuum-packed. Five regions of Finland were represented in these two pools. The samples of *Lactarius trivialis, Russula paludosa*, and *B. edulis* (500–1000 g) were obtained from two to four regions in Finland. Each subsample was separately lyophilized and vacuum-packed. Cultivated *A. bisporus* (200 g) were purchased from eight retail stores around Helsinki representing four major retail chains in Finland. The same amounts of each subsample (100 g) were weighed, pooled, lyophilized, and vacuum-packed. The lyophilized samples of each species were homogenized with a blender (Moulinex) just before vitamin D analysis.

Procedures. The ergocalciferol and 25-hydroxyergocalciferol contents were analyzed by HPLC, using internal standard methods. Cholecalciferol was used as an internal standard for ergocalciferol and 25-hydroxycholecalciferol as an internal standard for 25-hydroxyergocalciferol. The ergocalciferol results were also calculated, using an external standard method. In addition, the contribution of previtamin D_2 to the vitamin D activity of the mushrooms was approximated by taking into account that its extinction coefficient at 263–265 nm is about twice as low as that of ergocalciferol (Bell, 1978) and assuming that its biological activity is 35% that of ergocalciferol

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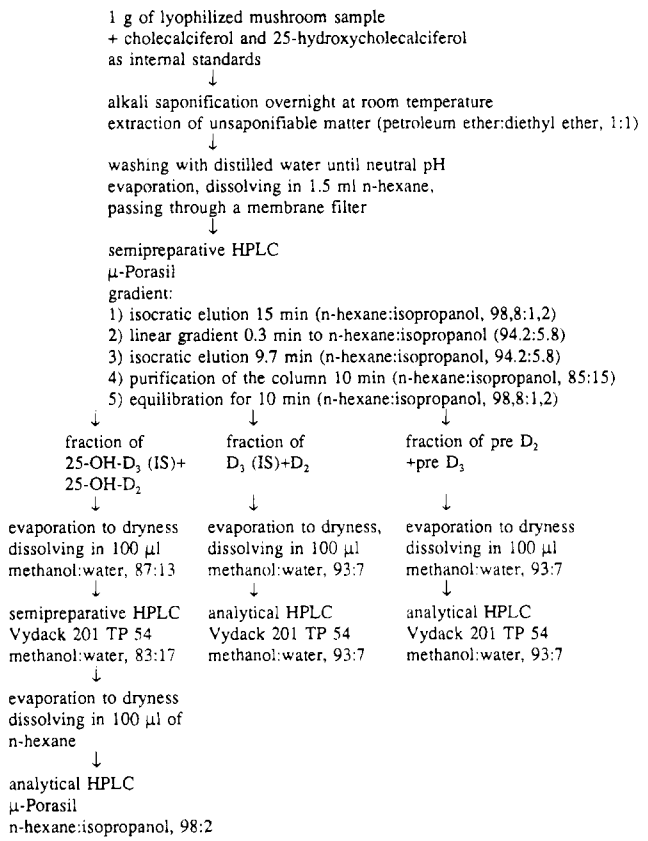

Figure 1. Scheme for procedure for determination of ergocalciferol, 25-hydroxyergocalciferol, and previtamin D_2 in mushroom samples.

(Hanewald et al., 1961; Zonta et al., 1982). A summary of the purification and quantification procedures is outlined in Figure 1.

The lyophilized and homogenized mushroom samples (1 g) were weighed in triplicate in 250-mL Erlenmeyer flasks. Thirty to 150 ng of 25-hydroxycholecalciferol and 40 ng–6 μg of cholecalciferol were added as internal standards. Blanks, in which no internal standards were added, were made for all mushroom species.

Saponification and extraction were performed as described earlier (Mattila et al., 1992). After saponification and extraction, the extracts were evaporated using Rotavapor, dissolved in 1.5 mL of *n*-hexane, and passed through a Millex HV membrane filter (0.45 μm, 1.5 cm; Millipore, France). Fractions containing previtamin D_2 and previtamin D_3, ergocalciferol and cholecalciferol, and 25-hydroxyergocalciferol and 25-hydroxycholecalciferol were collected during the straight-phase semipreparative HPLC step. The semipreparative cleanup equipment was the same as described earlier (Mattila et al., 1992). Gradient elution was used (Figure 1), and the injection volume was 1 mL of the sample extract. Under these conditions previtamin D_2 and previtamin D_3 as well as ergocalciferol and cholecalciferol eluted as one peak in about 8 and 12 min, respectively. On the other hand, 25-hydroxyergocalciferol and 25-hydroxycholecalciferol separated, and both compounds eluted in about 20–25 min. Collection times of 1.5 min before and after the retention times of the previtamins and ergo- and cholecalciferol standard peaks as well as 2 min before the retention time of the 25-hydroxyergocalciferol standard to 2 min after that of the 25-hydroxycholecalciferol standard were considered adequate for all samples. After collection, each fraction was analyzed separately.

The fractions containing previtamin D_2 and previtamin D_3 and ergocalciferol and cholecalciferol were evaporated to dryness under nitrogen and dissolved in 100 μL of 7% water in methanol. Both fractions were analyzed separately, using analytical reversed-phase HPLC with diode array detection at 264 nm, according to the method of Mattila et al. (1992; Figure 1); the injection volumes were 50 μL.

The fraction containing 25-hydroxyergocalciferol and 25-hydroxycholecalciferol was evaporated to dryness under nitrogen and dissolved in 100 μL of 13% water in methanol, and an injection volume of 80 μL was further purified with reversed-phase HPLC, according to the method of Mattila et al. (1993). Under these conditions 25-hydroxycholecalciferol and 25-hydroxyergocalciferol separated and eluted in about 20–25 min; the 25-hydroxycholecalciferol eluted first. The retention times of 25-hydroxyergocalciferol and 25-hydroxycholecalciferol were confirmed using standard compounds. The collections of the vitamin fractions were begun 2 min before the retention time of 25-hydroxycholecalciferol and finished 2 min after that of 25-hydroxyergocalciferol. The fraction containing these monohydroxylated metabolites was evaporated first under nitrogen and then continued in a vacuum oven at 30–40 °C until dryness. The residue was dissolved in 100 μL of *n*-hexane. Finally, an injection volume of 70 μL was analyzed, using a straight-phase HPLC system (Waters 486 UV detector set at 264 nm, Waters 600E controller with pump, Waters 700 satellite wisp autosampler, NEC power mate SX-16 computer, and μ-Porasil column). The mobile phase consisted of 98% *n*-hexane and 2% 2-propanol.

Method Reliability Tests. The reliability of the methods was tested by recovery and repeatability tests. The recovery tests were made by spiking mushroom samples with ergocalciferol (0.4–6 μg) and 25-hydroxyergocalciferol (83 and 166 ng) before saponification. The recoveries of added ergocalciferol varied from 86 to 109% (mean 98 ± 9.2%, $n = 4$), as calculated on the basis of the internal standard and from 82 to 95% (mean 88 ± 5.5%, $n = 4$) on the basis of the external standard. The recoveries for 25-hydroxyergocalciferol were 111 and 93% at the two spiking levels, as calculated on the basis of the internal standard. The repeatability tests were made by monitoring the coefficient of variation (CV%) of the triplicated samples. The average CV%, as calculated for the results given by the internal standard method, was 3 ± 3.5 ($n = 5$).

The purity and identity of the peaks of ergo- and cholecalciferol were checked, using the Hewlett-Packard diode array purity test system and by comparing the UV spectra of the ergocalciferol and cholecalciferol standards with the spectrum of the samples. For two mushroom samples, the purity of the peaks of ergo- and cholecalciferol was also checked by a tandem-column system in which two different reversed-phase columns (Zorbax ODS, 4.6 mm x 25 cm, and Vydack 201 TP 54, 5 μm, 25 cm x 4.6 mm) were connected together with a thin capillary tube.

The detection limits for ergocalciferol and cholecalciferol, defined as a signal 3 times the height of the noise level, were estimated to be 1.5 (diode array system) and 0.5 ng/injection (UV detection). The detection limits for 25-hydroxyergocalciferol and 25-hydroxycholecalciferol (UV detection) were 0.5 and 0.7 ng, respectively. The responses of the detectors used were linear for ergo- and cholecalciferol and their 25-hydroxy metabolites in the tested range of 3–700 ng/injection.

Moisture Analysis. To obtain moisture contents, samples of the mushrooms were weighed before and after freeze-drying. The residual moisture was determined by drying at 100 ± 2 °C to constant weight.

RESULTS AND DISCUSSION

In the chromatograms of the mushroom samples the ergocalciferol peak was symmetric and well-separated, as shown in Figures 2 and 3. The diode array system indicated good matching of the spectra of ergocalciferol standard versus ergocalciferol in the samples as well as high peak purity of ergocalciferol in all mushroom samples. In addition, good recoveries for added ergocalciferol were achieved (see Materials and Methods). Since the contents of ergocalciferol in *C. cibarius* and *C. tubaeformis* were high (12.8 and 29.82 μg/100 g of fresh weight, respectively; Table 1), further checking of the identity and purity was made for these mushroom

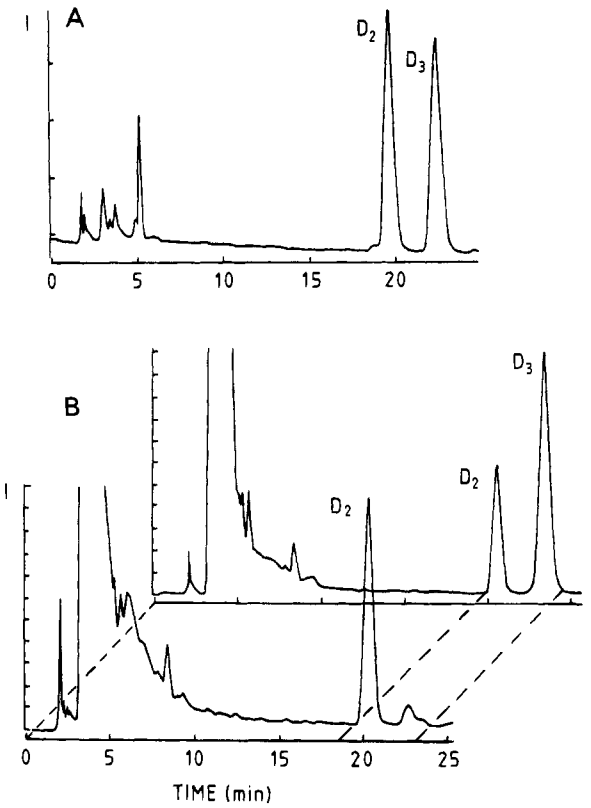

Figure 2. Analytical HPLC chromatograms of (A) a standard mixture of ergocalciferol and cholecalciferol and (B) a sample of *L. trivialis* with and without the internal standard (cholecalciferol).

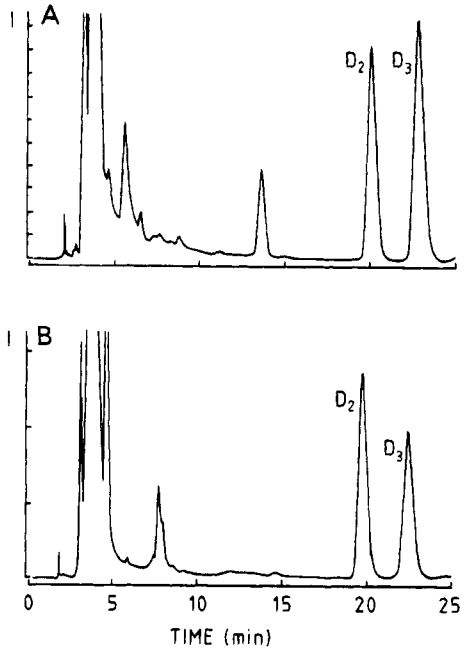

Figure 3. Analytical HPLC chromatograms of ergocalciferol and cholecalciferol in (A) a *C. cibarius* sample and (B) a *C. tubaeformis* sample.

samples. At the analytical HPLC step two reversed-phase columns connected with a thin capillary tube were used instead of one column, thereby increasing the separation capacity. However, one symmetric peak eluting at the same retention time as the ergocalciferol standard was obtained for the samples, and the area of the peak was the same as when using one column. The tests made confirmed the identity and purity of the ergocalciferol peak in the mushroom samples.

When ergocalciferol contents in mushrooms were determined, blanks with no added internal standards were made for all samples and showed that interfering compounds eluted at the same retention times as cholecalciferol (Figure 2). The peak area of these compounds varied 0–23.2% from the peak area of ergocalciferol in the samples. When ergocalciferol results were calculated using cholecalciferol as the internal standard and recoveries of cholecalciferol, the interfering compounds were taken into account by subtracting the peak area of the unknown compounds from the area of cholecalciferol. In addition, comparative calculations based on the external standard method were also made (Table 1). As shown in Table 1 the results obtained using the external and internal standard methods were quite similar. However, the variation of the triplicated samples was greater when the results were calculated on the basis of the external standard. In addition, the lower the recovery of the internal standard, the lower the results compared to the internal standard method obtained using the external standard method. If the recovery of the internal standard was near 100%, as in the case of *B. edulis* (Table 1), the results of ergocalciferol calculated on the basis of the external and internal standards were similar (3.1 and 2.91 μg/100 g, respectively). For *C. tubaeformis* sample 1 the recovery of the internal standard and the ergocalciferol result given by the external standard method were low (Table 1). In the case of this sample losses during purification steps might have happened. Because of the complexity of the vitamin D analysis, the internal standard method was preferred even if the subtraction systems as discussed above had to be used.

The ergocalciferol contents of the analyzed mushroom samples are listed in Table 1. The content of ergocalciferol was lowest in cultivated *A. bisporus* (0.21 μg/100 g) and highest in *C. tubaeformis* (29.82 ± 0.061 μg/100 g). The ergocalciferol contents in all of the other mushrooms varied from 2.91 to 12.8 μg/100 g.

Very little information is available on the ergocalciferol contents of the wild mushrooms analyzed in this study. To our knowledge only that for *C. cibarius* and *B. edulis* is available. Souci et al. (1986) give a content of 2.10 μg/100 g for *C. cibarius*, which is 5 times lower than in the present study (12.8 μg/100 g, Table 1). On the other hand, the ergocalciferol contents in *B. edulis* are of the same magnitude both in the present study (2.91 μg/100 g) and in the composition table of Souci et al. (1986; 3.10 μg/100 g).

Differences of climate, habitat, and degree of latitude may cause variation in ergocalciferol contents of mushrooms. Takamura et al. (1991) studied cultivated shiitake mushrooms and noticed a wide variation in ergocalciferol contents in different years. Most shiitake mushrooms are cultivated under natural climatic conditions. *C. cibarius* and *C. tubaeformis* contained very high amounts of ergocalciferol (12.8 and 29.82 μg/100 g, respectively), possibly due to the structural properties of the genus *Cantharellus*. Ono et al. (1976) studied conditions under which increased amounts of ergocalciferol could be formed from ergosterol in shiitake mushrooms by irradiation with a fluorescent sun lamp. They found that irradiation of the gill was about 10 times more effective than that of the pileus in producing ergocalciferol. Traits typical of the genus *Cantharellus* are that the fruiting body is quite thin and the pileus

Table 1. Ergocalciferol Contents in Mushroom Samples Using the Internal Standard (IS) and External Standard (ES) Methods, Recoveries of the Internal Standard (Cholecalciferol), and Dry Matter Contents

sample	dry matter, %	sample no.	IS method, μg/100 g of fresh wt	ES method, μg/100 g of fresh wt	recovery of IS
C. cibarius	7.1	1	12.97	12.22	84.4
		2	12.42	12.16	87.6
		3	12.95	10.20	70.4
		$\bar{x}$ =	12.8 ± 0.31	12 ± 1.1	81 ± 9.2
L. trivialis	5.8	1	5.14	4.52	82.8
		2	5.22	4.96	94.8
		3	6.08	5.74	88.8
		$\bar{x}$ =	5.5 ± 0.52	5.1 ± 0.62	89 ± 6.0
R. paludosa	6.0	1	5.69	5.76	90.6
		2	5.96	5.57	83.8
		3	5.73	4.80	79.4
		$\bar{x}$ =	5.8 ± 0.15	5.4 ± 0.51	85 ± 5.6
B. edulis	8.3	1	2.98	3.25	97.7
		2	2.93	3.12	95.5
		3	2.83	2.80	88.4
		$\bar{x}$ =	2.91 ± 0.076	3.1 ± 0.23	94 ± 4.9
C. tubaeformis	9.7	1	29.75	11.74	37.4
		2	29.87	26.89	84.1
		3	29.83	24.50	76.7
		$\bar{x}$ =	29.82 ± 0.061	21 ± 8.1	70 ± 25
A. bisporus	7.0	1		0.235	
		2	0.202	0.261	106.7
		3	0.212	0.248	96.0
		$\bar{x}$ =	0.21	0.25 ± 0.013	101

horny; therefore, the pileus and gill can very effectively be exposed to light as compared with others, e.g., *B. edulis*. The color of the mushroom might also affect the conversion of ergosterol via previtamin D_2 to ergocalciferol; *C. tubaeformis* is dark brown and might absorb light very effectively.

In the case of cultivated *A. bisporus* some figures are available in the literature. Souci et al. (1986) give a content of 1.94 μg/100 g (range 0.50–3.75 μg/100 g), Holland et al. (1991) 0 μg/100 g, and Sondergaard and Leerbeck (1982) <1 μg/100 g. These values are quite similar to the value obtained in the present study (0.21 μg/100 g). Cultivation, especially illumination conditions, may affect the ergocalciferol contents.

The 25-hydroxyergocalciferol contents were below the limit of detection (0.5 ng/injection) in all mushroom species. The contribution of previtamin D_2 to the total vitamin D activity of mushrooms was also screened, and it turned to be low, less than 10% of the contribution of ergocalciferol. In the mushroom samples no previtamin D_3 was detected, indicating that saponification at room temperature does not cause conversion of ergocalciferol and cholecalciferol to their previtamins.

In conclusion, about 90% of the total vitamin D content in the mushrooms studied was derived from ergocalciferol, whose content was remarkably high in wild mushrooms, especially in the genus *Cantharellus*. Since the annual consumption of mushrooms in Finland is only 0.61 kg/person (Statistics Finland, 1993), the average vitamin D intake from the mushrooms is consequently low. The significance of mushrooms may become emphasized for those individuals whose diets regurlarly contain an abundance of wild mushrooms.

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