

The ergocalciferol content of dried pigmented and albino *Cantharellus cibarius* fruit bodies

J. Ignacio RANGEL-CASTRO¹, Anders STAFFAS² and Eric DANELL^{1*}

¹ Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-750 07 Uppsala, Sweden.

² The National Food Administration, Box 622, SE-751 26 Uppsala, Sweden.

E-mail: Eric.Danell@mykopat.slu.se

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The ergocalciferol (vitamin D₂) content of individual fruit bodies of the edible mushroom *Cantharellus cibarius* (chanterelle) was measured with HPLC 2–6 years after drying. The concentrations of ergocalciferol in different fruit bodies varied between 0.12 and 6.30 µg g DW⁻¹, with a mean of 1.43 µg g DW⁻¹. This result indicates that dried and stored chanterelles contain significant amounts of ergocalciferol. The huge range in concentration challenges the validity of food tables based on means from pooled samples. The range may reflect differences in exposure to sun light, as fruit bodies within the same cluster showed little variation. Our study indicates that mycelial cultivation for ergocalciferol production is an interesting prospect if strains and incubation conditions are selected carefully. No significant difference in ergocalciferol concentration between pigmented and albino forms was found.

INTRODUCTION

The world consumption of chanterelle mushrooms (*Cantharellus* spp.) has been estimated at 150–200 km t yr⁻¹ (Watling 1997). Chanterelles are widely used in Europe, Africa, Asia and northwestern USA (Danell 1994), but knowledge about their biology is still scarce (Danell 1994, 1999). In Sweden, the per capita consumption of edible wild mushrooms of which *C. cibarius* is the most famous representative is 1 kg yr⁻¹ (Danell 1994). *C. cibarius* is a declining species in European forests (Arnolds 1995), but since this mushroom is ectomycorrhizal rather than saprotrophic (Danell 1999), it cannot be cultivated on compost or wood. Fruit body formation has been observed in the greenhouse where it formed ectomycorrhizal symbiosis with host seedlings in pots (Danell & Camacho 1997). Some nutritional studies have been made (Danell 1999), and strikingly high levels of ergocalciferol (vitamin D₂) were found by Mattila *et al.* (1994).

Ergocalciferol is synthesised from ergosterol under the action of UV-light (Budavari 1996b). Similarly, cholecalciferol (vitamin D₃) is synthesised from cholesterol in human skin. Ergocalciferol and cholecalciferol have the same metabolic activity in humans (Outila *et al.* 1999, Budavari 1996a), like hormones the hydroxy-

lated metabolites of these steroids regulates calcium transport.

To meet the demands of consumers, we decided to analyse fruit bodies that had been dried for 2–6 yr, since drying fruit bodies is an important method of storage. Our purpose was not to follow degradation with time, but to determine whether dried chanterelles contained significant amounts of vitamin D₂. In many studies the range in concentration between individual fruit bodies is not given, i.e. the mean value is only based on pooled samples. If the range in concentration varies several orders in magnitude, then mean values of food tables could be misleading. Since the range of ergocalciferol content among different fruit bodies was unknown, we wanted to study whether there was a variation due to geographical origin, and whether there was variation within clusters of fruit bodies. Since the production of ergocalciferol is light dependent, we wanted to test whether albino forms of *C. cibarius* have lower concentrations of ergocalciferol than normal forms, that contain yellow carotenoids situated on the fruit body surface (Arpin & Fiasson 1971, Gill & Steglich 1987, Mui, Feibelman & Bennett 1998).

MATERIALS AND METHODS

Eight Swedish fruit bodies of *Cantharellus cibarius* (*Cantharellaceae*) were collected from six forests. In Table 1, the geographical origin, collection year and

* Corresponding author.

Table 1. Sample number, geographical origin in Sweden, collection year, pigment variety, dry weight (DW) of analysed sample and content of ergocalciferol of the eight analysed *Cantharellus cibarius* specimens. The analytical variation (relative standard deviation) was 3%, based on six replicate measurements ($n = 6$).

Sample	Origin (lat. 56°–63° N)	Year	Pigment type	Amount analysed (g DW)	Ergocalciferol content in individual fruitbodies ($\mu\text{g g DW}^{-1}$)
N 3792	Ingarö	1998	Albino	1.0	1.44
N 3793	Ingarö	1998	Albino	1.0	1.37
N 3794	Olofström	1994	Albino	1.0	0.24
N 3795	Ramsele	1996	Albino	0.6	6.30
N 3796	Höör	1998	Albino	1.0	0.12
N 3797	Hökensås	1998	Carotenoid	1.0	0.21
N 3798	Hökensås	1998	Carotenoid	0.9	1.54
N 3799	Boo	1998	Carotenoid	1.0	0.21

Mean = 1.42 $\mu\text{g ergocalciferol g DW}^{-1}$.

pigment variety of the analysed *C. cibarius* specimens are given. The two samples from Ingarö originate from the same cluster of fruit bodies, while the two samples from Hökensås were collected 200 m apart within the same site. The other fruitbodies represent distant localities.

Drying is a common way of storing edible mushrooms in many parts of the world. Therefore the collected fruit bodies were dried for 12 h (+40 °C air stream) with a mushroom drier (Evermat, Bjurholms industriplast, Sweden) and stored in sealed plastic bags at room temperature in darkness for 2–6 yr before vitamin D analysis. This treatment is common practice among mushroom collectors.

The samples were analysed in March 2000 at the Swedish National Food Administration, using their routine HPLC method (Johnsson *et al.* 1989, Johnsson & Hessel 1987). The dried fruit bodies were individually ground in a mortar before weighing. 1 g was used for each measurement with two exceptions (Table 1). Each sample was mixed with 0.5 g ascorbic acid, 60 ml 99.5% ethanol, 10 ml of distilled water, 10 ml 50% potassium hydroxide solution, and 0.54–1.6 μg of cholecalciferol. Cholecalciferol was used as internal standard. The amount added depended on the expected amount of ergocalciferol in the sample. Each sample was saponified for 30 min. After the hydrolysis (95 °) was complete, 50 ml of distilled water was added through a reflux condenser. The sample was quantitatively transferred to a separation funnel, then 100 ml 40% ethanol was added, and the sample was extracted with 75 ml *n*-heptane. After separation of the phases, the heptane phase was transferred to a new separation funnel. The extraction procedure was repeated once. The combined heptane phases were washed once with 50 ml 1 M potassium hydroxide solution, then twice with 50 ml 40% ethanol, and finally with 50 ml portions of distilled water until the heptane phase was free of alkali. The sample was then evaporated to dryness, and dissolved in cyclohexane/*n*-heptane 1:1. The extract was cleaned with semi-preparative HPLC (Silica). Ergocalciferol and cholecalciferol co-elute on the silica column. The fraction taken was evaporated, and dissolved in acetonitrile/methanol 4:1. Finally, the

sample was analysed with quantitative HPLC (C-18). The sample content of ergocalciferol was calculated with the internal standard.

The analytical variation (relative standard deviation) was 3%, based on six replicate measurements ($n = 6$). Calculations were based on dry weight (DW), but to compare with the international standard $\mu\text{g 100 g fresh weight}^{-1}$ (FW) the results were compensated for a *C. cibarius* water content of 90% (Danell 1994).

RESULTS AND DISCUSSION

Our results indicate impressively high amounts of ergocalciferol in spite of the age of the *Cantharellus cibarius* fruit bodies (Table 1). Even though it is likely that the ergocalciferol content decreases over time, dry 2–6 yr old *C. cibarius* fruit bodies are clearly a good source for vitamin D₂. In Table 2 the values are expressed according to the international standard to compare with other literature data, and in comparison with cholecalciferol content in other food products. Both the average content of 14.2 $\mu\text{g 100 g FW}^{-1}$, and the maximum amount 63 $\mu\text{g 100 g FW}^{-1}$, show the potential of the species to become one of the richest dietary

Table 2. Vitamin D (D₂, ergocalciferol in mushrooms D₃, cholecalciferol in other products) in different food products.

Food product	Vitamin D content $\mu\text{g 100 g FW}^{-1}$
Chanterelle (<i>Cantharellus cibarius</i>)	14.2 (average) (range 1.2–63) (this study ¹) 12.8 (Mattila <i>et al.</i> 1994) 2.5 (SNFA ²)
Smoked eel (<i>Anguilla anguilla</i>)	36 (SNFA)
Sardines in oil (<i>Sardina pilchardus</i>)	15 (SNFA)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	13.6 (SNFA)
Enriched margarine	7.5 (SNFA)
Chicken	1.5 (SNFA)
Enriched 1.5% cow milk	0.38 (SNFA)
White button mushroom (<i>Agaricus bisporus</i>)	0.21 (Mattila <i>et al.</i> 1994) Not detectable (SNFA)

¹ The fresh weight (FW) value for chanterelle of this study was calculated from the dry weight (DW) value of Table 1, and a water content of 90%.

² SNFA, Swedish National Food Administration (1996).

sources of vitamin D₂. Today, vitamin D₂ is synthesised from UV-irradiation of ergosterol which is produced by special yeast strains (Budavari 1996c). Since techniques for mycelial cultivation of chanterelles are available (Danell 1994), chanterelle mycelia might be a source of vitamin D₂ with antirachitic and rodenticide applications.

The range in concentration is important when selecting genotypes and conditions for maximum yield in mycelial cultivation aimed at industrial ergocalciferol production. It is also important for understanding the physiology of the mushroom. Mattila *et al.* (1994) used a pool of 200 g of *C. cibarius*, so the range in ergocalciferol content among individual fruit-bodies due to geographical origin and light exposure was therefore lost.

In this study the range of ergocalciferol in *C. cibarius* was 0.12–6.3 µg g DW⁻¹. The difference between fruit bodies within the same cluster (Ingarö) was only 0.07 µg g DW⁻¹ (Table 1). However, within the same site (Hökensås) the difference was 1.33 µg g DW⁻¹ within a 200 m distance (Table 1). The large range might be due to variation in exposure of the fruit bodies to sunlight (shadow or clearing), exposure time (the longer photoperiod in early summer in the north), the surface/volume ratio (old or young fruit bodies), ergocalciferol synthesis induction, or a combination of these explanations. In our study, the two samples from Hökensås (Table 1) were dried and stored in the same way, at the same time, so the variation is probably not due to storage, but to the reasons mentioned above. The range in concentration, in addition to the mean value, is important to take into account when making food tables (Table 2). Takamura & Hoshino (1991) found both brand and annual differences in ergocalciferol concentration in *Lentinula edodes*. In our study only one specimen was collected in 1996, and it contained high amounts affecting our mean value. However, we focused on the range, although our mean is similar to that of Mattila *et al.* (1994).

Our field observations indicate a tendency for *C. cibarius* to grow in clearings, along paths or streams, where the chanterelles are exposed to light. Observations on cultivated fruit bodies indicate phototropism (Danell 1999). From this study, it is clear that the carotenoid pigments are not necessary for vitamin D synthesis, as albino varieties could transform ergosterol to ergocalciferol under the action of light to the same degree as pigmented morphs (Table 1). Carotenoids are quite rare in basidiomycetes (Gill & Steglich 1987) and those of *C. cibarius* are located on the fruit body surface (the flesh is white) and thoroughly expressed under light exposure; they may protect the fruit bodies against tissue damage caused by the oxidation of photosensitive compounds. Carotenoids as photoreceptors and antioxidants in fungi have been reviewed by Carlile (1970). In order to synthesise ergocalciferol, light is needed, but to prevent light-induced tissue damage carotenoids are needed. An antioxidative agent is particularly needed

for *C. cibarius* as it forms spores over a period of several weeks, in contrast to many other fleshy basidiomycetes which have a life span of only a few days (Danell 1994). According to Fraser (1995), vitamin D₂ is a rare form of vitamin D in nature. It is uncertain why *C. cibarius* fruit bodies contain such high amounts of ergocalciferol, since high amounts of the compound are used as a rodenticide (Fraser 1995, Budavari 1996b), and since cholecalciferol is known to cause hypercalcemia in mammalian, avian and fish kidneys (Das, Srivastava & Das 1990, Kenny *et al.* 1993, Soares, Kerr & Gray 1995). However, it is possible that these high amounts may explain why insects and gastropods rarely infest *C. cibarius* fruit bodies (Danell 1999).

Data about the toxicity levels of vitamin D vary between 50 µg d⁻¹ for children (i.e. 350 g FW of average chanterelles, or 50 average fruit bodies of 7 g FW each) to 250 or even 1875 µg d⁻¹ (i.e. 1760–13200 g FW average chanterelles, or 250–1885 average fruit bodies 7 g FW each) for adults (Parfitt 1999). The range in concentration rather than average concentration must be considered since 57 fruit bodies (7 g FW each) of the highest concentration we found may be a toxic dose in adults. We conclude that it is very difficult to eat enough chanterelles to reach toxic levels, especially over a period of several weeks. Over-eating of chanterelles may however cause other problems (Gerber 1989).

A rich source of ergocalciferol has also been found in *C. tubaeformis* (Mattila *et al.* 1994, Outila *et al.* 1999), a species which, according to rDNA-sequencing by Dahlman, Danell & Spatafora (2000), belongs to *Craterellus* within the *Cantharellaceae*. It is possible that highly appreciated wild mushrooms such as *Hydnum* spp. also contain high values of vitamin D₂ due to their close phylogenetic relationship to *Cantharellus* and *Craterellus* (Hibbett *et al.* 1997).

The RDA (Recommended Daily Allowance) values of vitamin D in Sweden are 5 µg d⁻¹ for adults, and 10 µg d⁻¹ for the elderly, children younger than 3 yr and pregnant women (Becker, Enghardt & Robertson 1994). The Swedish RDA for adults thus corresponds to 35 g of fresh average chanterelles. The same amount occurs in 2200 g of *Agaricus bisporus* (Mattila *et al.* 1994), may be since cultivated fruit bodies are grown in darkness. About five average chanterelle fruit bodies (7 g FW each) will generally contain the equivalent of the Swedish RDA of vitamin D, but in reality, according to this study 1–60 fruit bodies may be needed due to the range in ergocalciferol contents between individual fruit bodies. The bioavailability of ergocalciferol is 55–99% depending on the food source and individual, and from lyophilized and homogenized *Craterellus tubaeformis* it is well absorbed (Outila *et al.* 1999). In Sweden, 10% of the population gets less than half of the RDA of vitamin D (Becker 1994). This deficiency may be a contributing factor for osteoporosis and osteomalacia in some cases (Fraser 1995). Therefore, eating chanterelles, which can be stored in a dried condition for many years, is a much more appealing

supplement than eating cod liver oil, the classical source for vitamin D (Parfitt 1999).

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