

Distribution of free and esterified ergosterols in the medicinal fungus *Ganoderma lucidum*

Jian-Ping Yuan · Jiang-Hai Wang · Xin Liu

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Abstract The fruiting bodies, spores, and lipid from the spores of *Ganoderma lucidum* have been widely used for medicinal purpose in China. Ergosterol content may be a suitable marker for evaluating the quality of ganoderma spore and ganoderma spore lipid (GSL) products. A gradient reversed-phase high-performance liquid chromatography method was developed for the simultaneous determination of free and esterified ergosterols in *G. lucidum*. The contents of free and esterified ergosterols in the different parts (the stipe, pileus, tubes, and spores) of *G. lucidum* and GSL were determined. The results showed that total ergosterol levels in the stipe, pileus, tubes, and spores of *G. lucidum* were between 0.8 and 1.6 mg/g. The relative abundances of free to esterified ergosterol were different in the different parts of *G. lucidum*. The spores and the tubes, the hymenophore tissue that contains the spore-producing cells, have a considerably higher percentage of ergosteryl esters (41.9 and 39.7% of total ergosterol) in comparison with the pileus and stipe tissues (3.6 and 6.2%).

Keywords *Ganoderma lucidum* · Free and esterified ergosterols · Spores · Hymenophore · Stipe · Pileus

Introduction

Ganoderma lucidum is a medicinal fungus with a variety of bioactivities and has long been used and praised as the one

of the best folk remedies for its capability to promote health and longevity in China and other Oriental countries (Liu et al. 2005). Although the fruiting bodies have been utilized as medicine for several thousand years in China, the spores whose bioactivities might be much higher than that of the fruiting bodies of *G. lucidum* were realized and utilized only in the late twentieth century (Liu et al. 2002). The potential medicinal value and wide acceptability of *G. lucidum* have attracted intense interest in the search for pharmacological compounds from this edible fungus (Wasser and Weis 1999; Cheung et al. 2000). Numerous triterpene derivatives including highly oxygenated lanostane derivatives and common fungal steroids derived from ergosterol were previously isolated from *G. lucidum* (Rösecke and König 2000). A number of studies had showed that ergosterol and its peroxides might contribute to potential health benefits, including reducing pain related to inflammation, reducing the incident of cardiovascular disease, and inhibiting the tumor development (Bok et al. 1999; Yazawa et al. 2000; Takaku et al. 2001; Subbiah and Abplanalp 2003) by direct inhibition of angiogenesis (Takaku et al. 2001) and acting as an antioxidant and/or as anti-inflammatory agents (Zhang et al. 2002).

Sterol molecules are essential for maintaining the proper structure and function of eukaryotic cell membranes (Czub and Baginski 2006). Each living kingdom has selected its own naturally biosynthesized sterols, i.e., cholesterol in animals, β -sitosterol and other phytosterols in plants, and ergosterol in fungi (Suárez et al. 2002). Because of a strong correlation between ergosterol content and fungal dry mass (Pasanen et al. 1999), ergosterol has been used as a suitable marker for evaluating the fungal biomass in the oilseed canola (Abramson and Smith 2003), the wheat grains (Varga et al. 2006), the natural wetland (Headley et al. 2002),

J.-P. Yuan (✉) · J.-H. Wang · X. Liu
State Key Laboratory of Biocontrol/Food Engineering Research
Center of State Education Ministry, College of Life Sciences,
Sun Yat-Sen University,
Guangzhou 510275, People's Republic of China
e-mail: yuanjp@mail.sysu.edu.cn

tomato products (de Sio et al. 2000), atmospheric aerosols (Lau et al. 2006), etc.

Ergosterol exists in two forms: free ergosterol and ergosteryl esters (Fig. 1). Although ergosterol and ergosteryl esters had been isolated from the fruit bodies of *G. lucidum* (Gonzalez et al. 1999; Ziegenbein et al. 2006) and used as a suitable marker for evaluating the quality of ganoderma spore lipid (GSL) products extracted from the spores of *G. lucidum* (Yuan et al. 2006), little was known of the distribution of free and esterified ergosterols in *G. lucidum*.

A number of methods have been reported for the determination of ergosterol, but most studies to date have focused on the determination of free ergosterol, and the samples or extracts are first subjected to alkaline saponification before chromatographic analysis (Yuan et al. 2006, 2007). In our previous study, a gradient reversed-phase high-performance liquid chromatography (HPLC) method had been developed for the simultaneous determination of free ergosterol and ergosteryl esters in the medicinal fungus *Cordyceps sinensis* (Yuan et al. 2007). Although this method might be used for the analysis of the fruiting bodies of *G. lucidum*, it was not suitable for the analysis of the spores, whose components were more complicated than the fruiting bodies, especially the GSL samples, in which α -tocopherol acetate was added as an antioxidant during the manufacturing process (Yuan et al. 2006). Therefore, the aim of this work is to develop a new HPLC method capable of separating free ergosterol, ergosteryl esters, α -tocopherol acetate, and its saponified product α -tocopherol to avoid the effect of α -tocopherol acetate and α -tocopherol on the analysis of free ergosterol. The contents of free and esterified ergosterols in the stipe, pileus, tubes, spores, and GSL of *G. lucidum* and several commercial GSL soft capsule supplements were determined for the purpose of comparison. The direct HPLC analysis for the GSL sample solution and the saponification product is performed with little sample purification to prevent from unwanted sample losses (Abidi 2001).

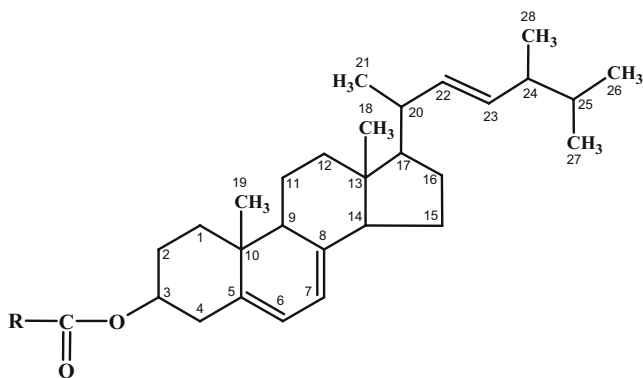


Fig. 1 Molecule structure of ergosterol ester

Materials and methods

Chemicals and reagents

HPLC-grade methanol was obtained from Merck KGaA (Darmstadt, Germany). HPLC-grade butanol was obtained from Ridel-deHaën (Seelze, Germany). HPLC-grade dichloromethane was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ergosterol and α -tocopherol acetate were obtained from Sigma-Aldrich Chemical (St. Louis, MO). Water was purified using a Millipore Simplicity system.

Sample preparation

G. lucidum was cultivated at a base located in a 1,000-m-high forested area in Fujian, China. The fungus was identified as *G. lucidum* (Curtis: Fr.) Karst., and the type specimen was deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences. The spores of *G. lucidum* were collected and then broken by using a crushing apparatus (Liu et al. 2005). The lipid in spores of *G. lucidum* was extracted on the industrial scale by using supercritical carbon dioxide (Hsu et al. 2001). Ethanol as a polar enhancer was added to elevate the effectiveness of extraction. In addition, four commercial GSL soft capsule supplements (XZ, ZK, HF, and HK) from four firms in China were purchased.

For GSL of *G. lucidum*, a 0.5-g amount of a sample was accurately weighed into a 10-ml volumetric flask and made up to volume with butanol. The samples were shaken by hand for 1 min and sampled for HPLC analysis.

For the saponification of GSL, a 0.5-g amount of GSL sample was accurately weighed into a 10-ml volumetric flask and dissolved in 5 ml of butanol. Then, 1 ml of freshly prepared methanolic NaOH at the concentration of 0.4 M was added and made up to volume with ethanol (Yuan et al. 2006). The sample was shaken by hand for 1 min and then left to stand at 25°C. During the saponification step, the reaction mixtures were sampled for HPLC analysis to monitor the progress of hydrolysis of ergosteryl esters.

For the sporoderm-broken spores and the fruiting bodies that were carefully divided into stipe, pileus (outer layers of the cap), and tube tissues (tube layer) and then were ground into powder and dried at 50°C, a 0.5-g amount of a sample was accurately weighed into 25-ml disposable centrifuge tubes containing 10 ml of the mixture of methanol and dichloromethane (75:25, v/v) followed by sonication for 10 min and centrifugation at 10,000×g for 5 min. The extraction procedure was repeated three times, and the total extracts were sampled for HPLC analysis.

For the commercial GSL soft capsule supplements, ten capsules from every sample were opened, and the inclu-

sions were thoroughly mixed. A 0.5-g amount of a sample was accurately weighed into a 10-ml volumetric flask and made up to volume with butanol. The samples were shaken by hand for 1 min and sampled for HPLC analysis.

HPLC method

HPLC was conducted on a Waters liquid chromatograph equipped with a 1525 binary pump and a 2996 photodiode array detector. Six reversed-phase columns from Waters (Milford, MA), Agilent Technologies (Santa Clara, CA), and Eka Chemicals (Bohus, Sweden), respectively, were tested to separate free ergosterol and ergosteryl esters in GSL at ambient temperature. Table 1 shows the main characteristics of these columns given by the manufacturers. The mobile phase consisted of solvent A (methanol/water, 80:20, v/v) and solvent B (methanol/dichloromethane, 75:25, v/v). The gradient procedure was used as follows: starting at sample injection, 0% of B for 5 min, a linear gradient from 0 to 100% of B for 30 min, 100% of B for 35 min. The flow rate was 1.0 ml/min. Chromatographic peak was identified by comparing the retention times and spectra against a known standard (Yuan et al. 2006, 2007). The detecting wavelength was set between 220 and 400 nm, and the chromatographic peaks were measured at a wavelength of 280 nm to facilitate the detection of free ergosterol and ergosteryl esters. Both free ergosterol and ergosteryl esters were quantified by area comparison with a free ergosterol standard. The sample was filtered through a 0.45- μ m filter. Aliquots of 20 μ l were injected into the HPLC for the determination. All injections were repeated three times.

Method validation

The stock standard of ergosterol was prepared at 300 μ g/ml, and additional calibration levels (300, 225, 150, 75, 37.5,

18.8, 9.4, 4.7, 2.4, 1.2, 0.6 μ g/ml) were prepared by a serial dilution with ethanol. The standard calibration curve was constructed using these ergosterol standard solutions. The linear regression analysis was carried out by plotting the peak areas against the concentrations of ergosterol. The linearity was demonstrated by a correlation coefficient (r^2) greater than 0.999. The limit of detection and the limit of quantification were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively.

The intraday and interday precisions for this HPLC method were determined by analyzing GSL sample on the same day and 3 different days, respectively. Precision was calculated as a relative standard deviation (RSD) for the repeated measurements. For recovery studies on added ergosterol, known volumes of ergosterol standard solutions were added to 0.5 g amount of GSL at four levels (1.0, 1.5, 2.0, and 3.0 mg/g). The spiked samples were dissolved in butanol after the described procedure. Background levels were subtracted in all recovery determinations.

Results

GSL, extracted from the spores of *G. lucidum*, is a complicated mixture of triglycerides and bioactive substances such as triterpenoids and sterols. In some of commercial GSL soft capsule supplements, α -tocopherol acetate is added as an antioxidant during the manufacturing process (Yuan et al. 2006). In the present study, six reversed-phase columns, coming from different manufacturers or with the packing materials of different surface areas, carbon load, and silanol activities were tested, and a mixed solvent of methanol, water, and dichloromethane as the mobile phase and the gradient elution procedure were used to separate free ergosterol, ergosteryl esters, α -tocopherol acetate, and other unknown compounds. The same mobile phase and chromatographic conditions were employed in all cases.

Table 1 Characteristics of the reversed-phase columns tested

Commercial brand	Column size (mm)	Particle size (μ m)	Surface area (m^2/g)	Pore size (\AA)	Carbon load (%)	Retention time (min)		
						Free ergosterol	Ergosteryl ester 1	Ergosteryl ester 2
Waters Symmetry [®] C18	250 \times 4.6	5	348	95	20.1	35.4	57.3	62.9; 64.6
Eka Kromasil 100-5-C18	250 \times 4.6	5	320	100	20	33.5	54.1	58.6; 59.4
Agilent ZORBAX Eclipse XDB-C18	250 \times 4.6	5	180	80	10	30.0	49.4	52.9
Waters SymmetryShield [™] RP18	250 \times 4.6	5	352	87	18.1	28.6	38.7	39.6
Waters XTerra [®] RP18	250 \times 4.6	5	170	141	14.8	26.1	36.9	37.6
Waters Nova-Pak C18	150 \times 3.9	4	120	60	7	22.9	36.7	37.6

The retention characteristics of free and esterified ergosterols on six reversed-phase columns are shown in Table 1.

The results show that the best peak shapes for these analytes in GSL were obtained using the ZORBAX Eclipse XDB-C18 column because there were less surface defects for the XDB-C18 materials (Chagolla et al. 2003). This combination of extradense surface coverage by the bonded phase and double endcapping produces a highly, deactivated stationary phase that virtually eliminates undesirable interactions between peaks 1 and 2 and the silica surface. As a result, superior peak shapes are assured (Fig. 2). Although the ZORBAX Eclipse XDB-C18 column, unlike Symmetry C18 and Kromasil 100-5-C18 columns, could not separate peaks 7 and 7' (ergosteryl ester 2) from each other, in the present study, the total content of ergosteryl esters was determined. Therefore, the ZORBAX Eclipse XDB-C18 column was used for the determination of ergosterol and ergosteryl esters in GSL.

Using a photodiode array detector, peaks were identified by taking the spectra of each peak during elution. The identification of free ergosterol was achieved by comparing its retention time and spectrum against the known standard. The spectra of ergosteryl esters are similar to that of free ergosterol, indicating that the esterification of ergosterol does not significantly change its UV absorptive character (Yuan et al. 2007). For the further identification of ergosteryl esters, the GSL solution was saponified for the hydrolysis of ergosteryl esters to produce free ergosterol. Sodium hydroxide (0.04 M) in reaction mixtures at ambient temperature was appropriate for hydrolysis of ergosteryl esters to avoid the production of flocculent precipitates in GSL (Yuan et al. 2006). In the present experiment, the complete hydrolysis of ergosteryl esters in GSL was achieved. The required least saponification time for almost complete hydrolysis of ergosteryl esters in GSL was about 15 h. During the saponification process, the reaction mixture was analyzed by HPLC to monitor the content change of free ergosterol and ergosteryl esters. The

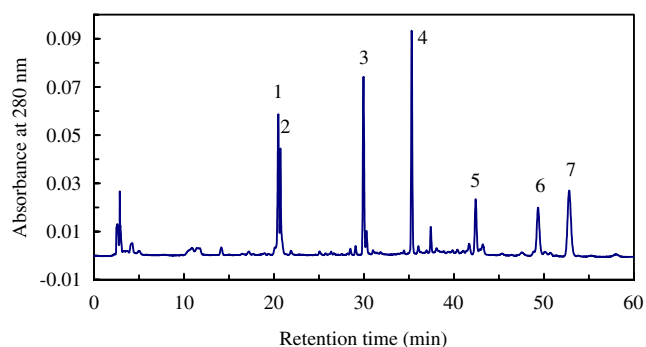


Fig. 2 Chromatogram of GSL of *G. lucidum* separated on the ZORBAX Eclipse XDB-C18 column using the mobile phase consisting of solvent A (methanol–water, 80:20, v/v) and solvent B (methanol–dichloromethane, 75:25, v/v). Peak identification: 1 unknown, 2 unknown, 3 ergosterol, 4 α -tocopherol acetate, 5 unknown, 6 ergosteryl ester 1, 7 ergosteryl ester 2

saponification product was directly injected for HPLC analysis. The contents of ergosteryl esters were measured by the comparison of the peak area with an ergosterol standard and quantitated as free ergosterol. The results showed that the concentrations of total ergosterol in reaction mixture kept unchanged (77.35 ± 0.42 mg/l) during the saponification process (Fig. 3). This result, at least on a gross scale, appears to support the validity of our assumption that the molar absorptivity of the ergosteryl esters is the same as the ergosterol.

Under the HPLC conditions tested, the standard calibration curve ($A=35,695C+16,359$, $r^2=0.9999$) was established by plotting the peak area (A) against the ergosterol concentration (C) in the range of 5–300 mg/l. The limit of detection and the limit of quantification for free ergosterol with a 20- μ l injection were 0.01 and 0.03 μ g/ml, respectively, corresponding to 0.2 and 0.6 ng injected on the column. The limit of detection and the limit of quantification for ergosteryl esters with a 20- μ l injection were 0.02 and 0.07 μ g/ml, respectively, corresponding to 0.4 and 1.4 ng injected on the column. This allows the detection of free ergosterol and ergosteryl esters in GSL samples at the concentrations as low as 0.2 and 0.4 μ g/g, respectively. Four replicate determinations of free ergosterol and ergosteryl esters 1 and 2 in the GSL sample were performed on the same day to evaluate intraday precision. For interday precision, 12 determinations were made on 3 different days. The RSD values for free ergosterol and ergosteryl esters were satisfactory (Table 2). For recovery, an ergosterol standard was spiked into 0.5 g of the GSL sample at the levels of 1.0, 1.5, 2.0, and 3.0 mg/g. The spiked samples were assayed and recoveries of ergosterol were found to be between 99.2 and 101.2%. The results obtained in the present study showed that the quantitative analysis of ergosterol and ergosteryl esters was feasible.

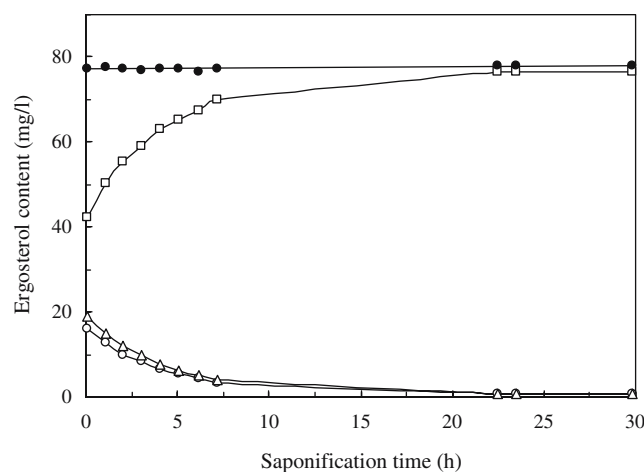


Fig. 3 Changes in the contents of free ergosterol (open squares), ergosterol esters 1 (open circles) and 2 (open triangles), and total ergosterol (filled circles) in the GSL solution during the saponification step with 0.04 M of NaOH in the reaction mixture

Table 2 Precision of the determination of free ergosterol and ergosteryl esters in intraday and interday analysis

Ergosterol (mg/g)	Intraday analysis ($n=4$)		Interday analysis ($n=12$)	
	Mean \pm SD	RSD (%)	Mean \pm SD	RSD (%)
Free ergosterol	1.281 \pm 0.011	0.9	1.278 \pm 0.012	0.9
Ergosteryl ester 1	0.561 \pm 0.008	1.4	0.564 \pm 0.009	1.6
Ergosteryl ester 2	0.537 \pm 0.007	1.3	0.553 \pm 0.010	1.8

Free ergosterol and ergosteryl esters in the stipe, pileus, tubes, and spores of *G. lucidum* were analyzed using the developed HPLC method. The contents of free ergosterol and ergosteryl esters are shown in Table 3. The results showed that *G. lucidum* contained both free and esterified ergosterols and their distribution varied in different parts of *G. lucidum*. Total ergosterol contents in different parts ranged from 0.8 to 1.6 mg/g dry matter. The relative abundances of free to esterified ergosterol were also different in the different parts of *G. lucidum*. As can be seen from Table 3, the contents of esterified ergosterol were much higher in the tubes and the spores than the pileus and stipe. While the contents of free ergosterol in the stipe (0.781 mg/g) and the pileus (1.550 mg/g) are higher than that in the spores (0.499 mg/g) and the tubes (0.653 mg/g), the contents of esterified ergosterol in the spores (0.360 mg/g) and the tubes (0.430 mg/g) are significantly higher than that in the stipe (0.052 mg/g) and the pileus (0.058 mg/g) of *G. lucidum*. The esterified ergosterol contents constitute 3.6, 6.2, 39.7, and 41.9% of the total ergosterol in the pileus, stipe, tubes, and spores of *G. lucidum*, respectively.

Based on the above results, the contents of free ergosterol and ergosteryl esters in GSL from spores and four commercial GSL soft capsule samples XZ, ZK, HF, and HK were analyzed and compared with our previous results in the contents of free ergosterol and total ergosterol (Yuan et al. 2006). The results showed that the content of free ergosterol in sample XZ was lower while the content of

total ergosterol was in accordance with our previous results (Yuan et al. 2006). As shown in Table 3, GSL from spores contained 1.117 mg/g of ergosteryl esters and 2.395 mg/g of total ergosterol. In contrast, only a small quantity of ergosterol (samples ZK and HF) or no ergosterol (sample HK) was found in the commercial GSL samples with the exception of sample XZ, which was in accordance with our previous results (Yuan et al. 2006). The percentages of esterified ergosterol in all GSL samples are higher (46.6 to 75.1%) than that in the spores (41.9%). Therefore, the relative abundances of free to esterified ergosterol in GSL could not represent that in ganoderma spores.

Discussion

As can be seen from Table 3, the contents of free and esterified ergosterols in different parts of *G. lucidum* show significant differences, indicating that the pileus, the tubes, and the stipe may have different physiological functions for the growth and multiplication of *G. lucidum*. The spores and the tubes, the hymenophore tissues that contain the spore-producing cell and abundant spores, tend to have higher concentration of ergosterol esters than the pileus and the stipe. It has been reported that the contents of ergosteryl esters increase steadily during ascus development and sporulation in *Saccharomyces cerevisiae* (Illingworth et al. 1973).

Table 3 Contents of free, esterified, and total ergosterols ($n=3$)

Sample	Free ergosterol (mg/g)	Ergosteryl esters (mg/g) ^a	Total ergosterol (mg/g) ^{a, b}	Esterified ergosterol (%) ^c
Pileus	1.550 \pm 0.031	0.058 \pm 0.007	1.608	3.6
Stipe	0.781 \pm 0.009	0.052 \pm 0.004	0.833	6.2
Tubes	0.653 \pm 0.018	0.430 \pm 0.016	1.083	39.7
Spores	0.499 \pm 0.012	0.360 \pm 0.012	0.859	41.9
GSL	1.278 \pm 0.017	1.117 \pm 0.019	2.395	46.6
XZ	0.557 \pm 0.011	1.193 \pm 0.012	1.750	68.2
ZK	0.093 \pm 0.004	0.119 \pm 0.005	0.212	56.1
HF	0.048 \pm 0.003	0.145 \pm 0.005	0.193	75.1
HK	<detection limit	<detection limit	–	–

^a Ergosteryl esters were quantitated as free ergosterol.

^b The “Total ergosterol” column is the total of the “Free ergosterol” and “Ergosteryl esters” columns.

^c The “Esterified ergosterol (%)” column is 100 multiplied by the “Ergosteryl esters” column divided by the “Total ergosterol” column.

Ergosterol has been shown to be esterified to long-chain fatty acids, predominately to unsaturated C₁₆ and C₁₈ fatty acids, during fungal cellular growth (Bailey and Parks 1975). In fungal cells, the synthesis, storage, and hydrolysis of ergosteryl esters occur in different subcellular compartments. Esterification of ergosterol occurs in the endoplasmic reticulum, whereas hydrolysis of ergosteryl esters takes place in secretory vesicles and in the plasma membrane (Zinser et al. 1993). Because free ergosterol is a component of the membrane, whereas ergosteryl esters are extramembranous lipids, esterification can also serve as a means of regulating the amount of ergosterol in the membrane (Taylor and Parks 1978). Ongoing membrane proliferation may be a driving force for the release of ergosterol from ergosteryl esters (Zinser et al. 1993; Leber et al. 1995). Therefore, the ergosteryl ester pool functions as an expandable reserve of free ergosterol for membrane biosynthesis. This reserve is utilized not only when the cell cannot synthesize more ergosterol (anaerobic conditions) but also when the cell is growing rapidly (aerobic conditions; Taylor and Parks 1978).

The membrane lipid ergosterol is found almost exclusively in fungi and is frequently used as an indicator of living fungal biomass based on the assumption that ergosterol is labile and therefore rapidly degraded upon death of fungal hyphae (Mille-Lindblom et al. 2004; Görs et al. 2007). However, recent studies suggested that ergosterol content reflected both living and dead fungal mass (Mille-Lindblom et al. 2004; Zhao et al. 2005; Lensing and Wise 2007). Mille-Lindblom et al. (2004) found that ergosterol could be present at appreciable concentrations and for considerable time in the absence of living fungi, and the degradation of ergosterol was very slow. They suggested that ergosterol should be used cautiously as a biomarker for living fungi, and the possibility that a significant fraction of the measured ergosterol did not represent living fungal biomass should be considered (Mille-Lindblom et al. 2004).

However, these studies did not differentiate two different forms of ergosterol, i.e., free ergosterol from the fungal cell membrane and ergosteryl esters from the cytosolic lipid particles. Most studies have focused on the determination of free ergosterol, and thus only the total ergosterol content could be estimated. We suggest that it is free ergosterol (not total ergosterol) that provides a better quantitative measure of fungal cells and should be used as a biomarker for fungal biomass.

As can be seen from Table 3, GSL from spores contains a higher content of total ergosterol (2.395 mg/g) in comparison with spores (0.859 mg/g). Herein, the lipid content in spores of *G. lucidum* is estimated to be about 35.9%. It has been reported that 37.5 g of GSL might be obtained from 100 g of spores by the supercritical carbon

dioxide extraction (Liu et al. 2002, 2005; Yuan et al. 2006). In GSL, both free and esterified ergosterols are dissolved in triglycerides. It is possible that a small quantity of free ergosterol is esterified during the storage of GSL. Therefore, the percentages of esterified ergosterol in all GSL samples are higher than that in the spores.

As shown in Table 3, the contents of free ergosterol and ergosteryl esters in the commercial GSL soft capsule samples have significant differences. The reason for this might be that some of commercial GSL soft capsule samples did not consist of pure GSL and might be a mixture of GSL and vegetable oil (Yuan et al. 2006), in which no ergosterol is found (Kalo and Kuuranne 2001; Abidi 2004; Jimenez-Escrig et al. 2006). Ergosterol is a membrane component of most fungi but is absent from vascular plants and metazoan animals (Charcosset and Chauvet 2001). The chromatographic profiles of some vegetable oils were complicated in comparison with pure GSL. Although ergosterol only is a portion of the bioactive constituents in GSL, the content of free ergosterol and ergosteryl esters may be a suitable marker for evaluating the quality of the commercial GSL soft capsule supplements and ganoderma spore products.

The dominant fatty acids in yeast steryl esters have been reported to be unsaturated C₁₆ and C₁₈ fatty acids (Bailey and Parks 1975). However, in the present study, the major fatty acids in ergosteryl esters produced by *G. lucidum* were not identified. The structures of these ergosteryl esters should be further identified by using liquid chromatography–mass spectrometry (MS), magnetic resonance imaging, or gas chromatography–MS in the future.

References

- Abidi SL (2001) Chromatographic analysis of plant sterols in foods and vegetable oils. *J Chromatogr A* 935:173–201
- Abidi SL (2004) Capillary electrochromatography of sterols and related steryl esters derived from vegetable oils. *J Chromatogr A* 1059:199–208
- Abramson D, Smith DM (2003) Determination of ergosterol in canola (*Brassica napus* L.) by liquid chromatography. *J Stored Prod Res* 39:185–191
- Bailey RB, Parks LW (1975) Yeast sterol esters and their relationship to the growth of yeast. *J Bacteriol* 124:606–612
- Bok JW, Lerner L, Chilton J, Klingeman HG, Towers GHN (1999) Antitumor sterols from the mycelia of *Cordyceps sinensis*. *Phytochemistry* 51:891–898
- Chagolla D, Ezedine G, Ba Y (2003) Solvation, interaction and dynamics of xenon atoms in HPLC column materials studied by variable-temperature dependent Xe-129, H-1-Xe-129 cross-polarization, and two-dimensional exchange NMR experiments. *Micropor Mesopor Mat* 64:155–163
- Charcosset JY, Chauvet E (2001) Effect of culture conditions on ergosterol as an indicator of biomass in the aquatic hyphomycetes. *Appl Environ Microb* 67:2051–2055

- Cheung WMW, Hui WS, Chu PWK, Chiu SW, Ip NY (2000) Ganoderma extract activates MAP kinases and induces the neuronal differentiation of rat pheochromocytoma PC12 cells. *FEBS Lett* 486:291–296
- Czub J, Baginski MJ (2006) Comparative molecular dynamics study of lipid membranes containing cholesterol and ergosterol. *Biophys J* 90:2368–2382
- de Sio F, Laratta B, Giovane A, Quagliuolo L, Castaldo D, Servillo L (2000) Analysis of free and esterified ergosterol in tomato products. *J Agri Food Chem* 48:780–784
- Gonzalez AG, Leon F, Rivera A, Munoz CM, Bermejo J (1999) Lanostanoid triterpenes from *Ganoderma lucidum*. *J Nat Prod* 62:1700–1701
- Görs S, Schumann R, Häubner N, Karsten U (2007) Fungal and algal biomass in biofilms on artificial surfaces quantified by ergosterol and chlorophyll a as biomarkers. *Int Biodeterior Biodegrad* 60:50–59
- Headley JV, Peru KM, Verma B, Robarts RD (2002) Mass spectrometric determination of ergosterol in a prairie natural wetland. *J Chromatogr A* 958:149–156
- Hsu RC, Lin BH, Chen CW (2001) The study of supercritical carbon dioxide extraction for *Ganoderma lucidum*. *Ind Eng Chem Res* 40:4478–4481
- Illingworth RF, Rose AH, Beckett A (1973) Changes in the lipid composition and fine structure of *Saccharomyces cerevisiae* during ascus formation. *J Bacteriol* 113:373–386
- Jimenez-Escrig A, Santos-Hidalgo AB, Saura-Calixto F (2006) Common sources and estimated intake of plant sterols in the Spanish diet. *J Agric Food Chem* 54:3462–3471
- Kalo P, Kuورانne T (2001) Analysis of free and esterified sterols in fats and oils by flash chromatography, gas chromatography and electrospray tandem mass spectrometry. *J Chromatogr A* 935:237–248
- Lau APS, Lee AKY, Chan CK, Fang M (2006) Ergosterol as a biomarker for the quantification of the fungal biomass in atmospheric aerosols. *Atmos Environ* 40:249–259
- Leber R, Zinser E, Hrastnik C, Paltauf F, Daum G (1995) Export of sterol esters from lipid particles and release of free sterols in the yeast, *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 1234:119–126
- Lensing JR, Wise DH (2007) Impact of changes in rainfall amounts predicted by climate-change models on decomposition in a deciduous forest. *Appl Soil Ecol* 35:523–534
- Liu X, Yuan JP, Chung CK, Chen XJ (2002) Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*. *Cancer Lett* 182:155–161
- Liu X, Wang JH, Yuan JP (2005) Pharmacological and anti-tumor activities of *Ganoderma* spores processed by top-down approaches. *J Nanosci Nanotechnol* 5:2001–2013
- Mille-Lindblom C, von Wachenfeldt E, Tranvik LJ (2004) Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. *J Microbiol Methods* 59:253–262
- Pasanen AL, Yli-Pietila K, Pasanen P, Kalliokoski P, Tarhanen J (1999) Ergosterol content in various fungal species and biocontaminated building materials. *Appl Environ Microb* 65:138–142
- Rösecke J, König WA (2000) Constituents of various wood-rotting basidiomycetes. *Phytochemistry* 54:603–610
- Suárez Y, Fernández C, Ledo B, Ferruelo AJ, Martín M, Vega MA, Gómez-Coronado D, Lasunción MA (2002) Differential effects of ergosterol and cholesterol on Cdk1 activation and SRE-driven transcription. Sterol stringency for cell cycle progression in human cells. *Eur J Biochem* 269:1761–1771
- Subbiah MTR, Abplanalp W (2003) Ergosterol (major sterol of baker's and brewer's yeast extracts) inhibits the growth of human breast cancer cells in vitro and the potential role of its oxidation products. *Int J Vitam Nutr Res* 73:19–23
- Takaku T, Kimura Y, Okuda H (2001) Isolation of an antitumor compound from *Agaricus blazei* murill and its mechanism of action. *J Nutr* 131:1409–1413
- Taylor FR, Parks LW (1978) Metabolic interconversion of free sterols and sterol esters in *Saccharomyces cerevisiae*. *J Bacteriol* 136:531–537
- Varga M, Bartók T, Mesterházy A (2006) Determination of ergosterol in *Fusarium*-infected wheat by liquid chromatography–atmospheric pressure photoionization mass spectrometry. *J Chromatogr A* 1103:278–283
- Wasser SP, Weis AL (1999) Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective. *Crit Rev Immunol* 19:65–96
- Yazawa Y, Yokota M, Sugiyama K (2000) Antitumor promoting effect of an active component of polyporus, ergosterol and related compounds on rat urinary bladder carcinogenesis in a short-term test with concanavalin A. *Biol Pharm Bull* 23:1298–1302
- Yuan JP, Wang JH, Liu X, Kuang HC, Huang XN (2006) Determination of ergosterol in ganoderma spore lipid from the germinating spores of *Ganoderma lucidum* by high-performance liquid chromatography. *J Agri Food Chem* 54:6172–6176
- Yuan JP, Wang JH, Liu X, Kuang HC, Zhao SY (2007) Simultaneous determination of free ergosterol and ergosterol esters in *Cordyceps sinensis* by HPLC. *Food Chem* 105:1755–1759
- Zhang YJ, Mills GL, Nair MG (2002) Cyclooxygenase inhibitory and antioxidant compounds from the mycelia of the edible mushroom *Grifola frondosa*. *J Agri Food Chem* 50:7581–7585
- Zhao XR, Lin Q, Brookes PC (2005) Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? *Soil Biol Biochem* 37:311–317
- Ziegenbein FC, Hanssen HP, König WA (2006) Secondary metabolites from *Ganoderma lucidum* and *Spongiporus leucomallellus*. *Phytochemistry* 67:202–211
- Zinser E, Paltauf F, Daum G (1993) Sterol composition of yeast organelle membranes and subcellular distribution of enzymes involved in sterol metabolism. *J Bacteriol* 175:2853–2858