Microwave-Assisted Extraction of the Fungal Metabolite Ergosterol and Total Fatty Acids†

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A novel microwave-assisted extraction (MAE) technique for isolating the fungal metabolite ergosterol was developed and applied to fungal hyphae and spores, mushrooms, filtered air, artificially contaminated corn, naturally contaminated grain dust, and soil. The procedure involves irradiation of milligram-sized samples in a conventional microwave oven for 35 s in the presence of methanol and aqueous sodium hydroxide and results in simultaneous extraction and saponification. Total ergosterol was determined by reversed phase high-performance liquid chromatography with ultraviolet detection at 282 nm and confirmed by gas chromatography–mass spectrometry (GC–MS). Results from a variety of fungal samples showed MAE-derived ergosterol values to be comparable with those obtained by classical solvent extraction and to significantly exceed those obtained by supercritical fluid extraction (SFE). Total fatty acid profiles of spores were determined (by GC–MS) on the same extracts as used for ergosterol analyses. The MAE procedure is simple, rapid, reliable, and economical with respect to amounts of reagents required, especially when compared with classical solvent and SFE.

Keywords: *Ergosterol; extraction; microwave-assisted; fungi; fatty acids; analysis*

INTRODUCTION

Ergosterol, in both free and conjugated forms, is the major sterol constituent of most fungi and is either absent from or only a minor constituent in higher plants (Weete, 1980c). Seitz et al. (1977) suggested that this metabolite could be used as an indicator of fungal contamination. Since then, it has become widely used for such a purpose in a diversity of matrices (Young and Games, 1993; Newell, 1992).

The methods used most frequently for analysis of ergosterol [cf. Young and Games (1993)] are based on that of Seitz et al. (1977, 1979) and involve methanolic extraction, alkaline saponification, pentane extraction, and reversed phase high-performance liquid chromatographic (HPLC) separation with ultraviolet (UV) detection at 282 nm. Reported modifications include combined methanolic extraction and saponification (Newell et al., 1988; Zill et al., 1988; Schwadorf and Mueller, 1989) or the use of CO_2 supercritical fluid extraction (SFE) and supercritical fluid chromatography (Young and Games, 1993).

The organic solvent based methods of extraction, hereafter referred to as “classical”, typically require large samples (e.g. up to 20 g) and large volumes of reagents (up to 300 mL) and are labor intensive (at least a dozen manipulations per sample) and lengthy (may take in excess of an hour) [see Schwadorf and Mueller (1989) and citations therein]. The SFE-based method overcomes many of these objections but requires expensive equipment. In the course of requiring a method for the extraction and analysis of ergosterol from many small (microgram) samples and discovering certain limitations to SFE for a particular application, alternative methods of extraction were investigated.

Microwave energy has been shown to greatly accelerate (up to 240-fold) a wide variety of chemical reactions (Gedye et al., 1986; Giguerre et al., 1986; Mingos and Baghurst, 1991) and extractions of organic chemicals from a variety of matrices (Ganzler et al., 1986; Onuska and Terry, 1993; Lopez-Avila et al., 1994). Prior demonstration of the utility of combined solvent extraction and saponification for ergosterol (Newell et al., 1988; Zill et al., 1988; Schwadorf and Mueller, 1989) as well as the hydrolysis of esters by microwave irradiation (Dayal et al., 1991) and microwave digestion for the determination of phenolic acids in plant cell walls (Provan et al., 1994) suggested that microwave technology might be applicable to the extraction/saponification of ergosterol. This paper describes the development of a microwave-assisted extraction (MAE) technique and its application to the analysis of ergosterol in samples of pure fungi or samples contaminated by them. This method of extraction was also extended to the analysis of total fatty acid (FA) profiles of fungal spores.

EXPERIMENTAL PROCEDURES

Reagents. All reagents and solvents were of analytical reagent grade. Ergosterol was recrystallized from ethanol.

Samples. Using sterile techniques, fungal spores (see Table 1) were grown on a 2% malt agar medium until the entire plate was covered (4 days at 23–25 °C). In addition, spores of some common fungal species encountered in another study (see Table 2) were collected, subcultured, and propagated (10 days at 23 °C) on sterile rice according to the procedure of Murad et al. (1993). The resulting spores were harvested by vacuum or by washing the rice with water, filtering, and drying the filter in a vacuum oven at ambient temperature.

The plates were then washed off with sterile water or the filters scraped off and the spores transferred to a screw cap test tube containing sterile water. One drop of Tween 20 was added to the suspension, which was sonicated for 10 min to separate clumps of spores. Densities of spores were determined by counting spores in a hemacytometer and aliquots containing the desired number of spores (500–25 000) taken for extraction.

Hyphae of *Alternaria alternata* were collected after being grown on 2% malt agar.

Mushrooms (*Agaricus bisporus*) were obtained from a local grocery store, frozen in liquid nitrogen, ground, and then freeze-dried.

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Table 1. Levels of Ergosterol in Various Fungi As Determined by Microwave-Assisted, Classical, and Carbon Dioxide Supercritical Fluid Extractions

	ergosterol[a]							
	MAE[b]		classical[c]		SFE[d]		SFE-M[e]	
sample	concn	%RSD	concn	%RSD	concn	%RSD	concn	%RSD
A. bisporus caps	6870[f]	4.0	4090	10.7	395	27.6	1379	27.8
A. alternata hyphae	898	4.4	918	6.4	82.4	16.3	369.2	3.6
moldy bread	521.1	4.8	492.0	4.0			86.4	8.2
moldy corn	92.9	2.8	107.4	10.7				
A. alternata spores[g]	120[h]		65.3				28.6	10.1
C. cladosporioides spores[g]	6.27	8.5	6.27	13.7	2.94	10.1		
Penicillium olsonii spores[g]	2.66	7.6	2.19	9.9				

[a] Ergosterol determined by HPLC with UV detection at 282 nm. Values are averages of triplicate extractions. [b] Microwave-assisted extraction: samples were treated with 2 mL of methanol and 0.5 mL of aqueous 2 M sodium hydroxide and microwaved for 35 s at 50% power, then neutralized and extracted with pentane. [c] Classical extraction: samples were blended in methanol, centrifuged, refluxed for 30 min in ethanolic potassium hydroxide, and extracted with pentane. [d] Carbon dioxide supercritical fluid extraction: for 10 min at a density of 0.90 g/mL. [e] Same as *d* except that samples were pretreated with 250 μL of methanol. [f] Ergosterol concentration in μg/g. [g] Sample size ca. 25 000 spores. [h] Ergosterol concentration in pg/spore.

Table 2. Correlation of Ergosterol Levels with Volume and Surface Area of Various Fungi

spore species	volume[a] (μm^3/spore)	surface area[a] (μm^2/spore)	ergosterol pg/spore[b]	ergosterol fg/μm^3	ergosterol fg/μm^2
A. alternata	2150	850	120	55.8	141
C. cladosporioides	45	57.5	6.27	139	109
P. olsonii	9.9	22.3	2.66	269	119

[a] Average maximum and minimum spore dimensions determined for 100 spores; volume and surface areas calculated on the basis of a prolate spheroid. [b] Ergosterol determined by microwave-assisted extraction and HPLC with UV detection at 282 nm. Values are averages of triplicate extractions.

Moldy bread was obtained by placing several slices of several-day-old bread into a clear plastic bag and allowing the bread to stand for several weeks at room temperature until it was well covered with mold; the bread was then air-dried, ground into a powder, and sieved, and only that material passing through a 35 mesh screen (<500 μm) was used.

Field corn naturally contaminated with *Fusarium graminearum* was dried and ground. Silks of field corn that had been inoculated with various *Fusarium* sp. were collected, macerated in sterile water, and freeze-dried.

As part of a study on the influence of fungal spores on air quality, samples of air (1−3 m^3) from 400 homes were passed through filters, which collected all air-suspended particles.

Samples of cultivated sandy, loam, and clay soils from the fields at the Central Experimental Farm in Ottawa, an undisturbed native forest soil from Winchester, ON, and an undisturbed native grassland soil from Swift Current, SK, were collected, air-dried, and passed through a 20 mesh sieve.

Grain dust was collected from a variety of grain elevators across Canada.

Microwave-Assisted Extraction. Samples (ca. 10−100 mg of fungal hyphae, moldy bread, corn, and grain dust, 500 mg of soil, ca. 500−25 000 spores of fungi, or an air filter) were suspended in 2.0 mL of methanol, placed into a 17 mL culture tube, treated with 0.5 mL of 2 M aqueous sodium hydroxide, and tightly sealed with a Teflon-lined screw cap. The culture tubes were then placed within screw-capped 250 mL plastic bottles and tightly sealed. This combination was then placed at the center of a domestic microwave oven (Sears Kenmore, Model 88592, Sears Canada Inc., Toronto, ON) operating at 2450 MHz and 750 W maximum output and heated at 50% power for 35 s. After sufficient time for the samples to cool, ca. 15 min, the culture tubes were removed from the plastic outer bottle. The contents were neutralized with 1 M aqueous hydrochloric acid and then extracted with pentane (3 × ca. 2 mL), all within the culture tube. The combined pentane extracts were evaporated to dryness and then made to the appropriate volume (100−1000 μL) in methanol prior to analysis.

Note: In some subsequent applications of this method involving larger sample sizes (e.g. 250 mg), tube ruptures became more frequent. The problem was overcome by microwaving in 27 mL culture tubes for 20 s and, after several minutes, an additional 15 s.

Classical Extraction. Samples, typically ca. 10−100 mg or ca. 25 000 spores, were extracted in methanol, and the extract was treated with ethanol and potassium hydroxide and refluxed for 30 min, cooled, diluted with water, and extracted with pentane. Details of this procedure are given elsewhere (Miller et al., 1983). The combined pentane extracts were evaporated to dryness and then made to the appropriate volume in methanol prior to analysis.

Supercritical Fluid Extraction. Samples, typically ca. 10−250 mg or ca. 25 000 spores on filter paper, in a 7 mL stainless steel extraction thimble, were placed into a Hewlett-Packard 7680A SFE module (Hewlett-Packard Co., Palo Alto, CA) and extracted under the following operating conditions: extraction chamber temperature, 40 °C; extraction conducted with supercritical CO_2 at a density of 0.90 g/mL (pressure 281 bar) for 5 min in the static mode and then 10 min in the dynamic mode at a flow rate of 3.3 mL/min; analytes from the extraction chamber were trapped on an octadecylsilane (ODS) column (ca. 5 × 55 mm) at 40 °C; the trap was then heated to 50 °C and rinsed successively with 0.5, 1.0, and 1.5 mL of methanol. Ergosterol was found in the 1.0 mL fraction. In some instances, the filter papers were treated with 250 μL of methanol prior to SFE.

High-Performance Liquid Chromatographic Analysis. Generally, separations were achieved on a 150 × 4.6 mm stainless steel column packed with ODS 3 μm (CSC, Montreal, PQ) and eluted with methanol/water (95:5) at a flow rate of 2 mL/min. Subsequently, acetonitrile/methanol (80:20) at a flow rate of 1.3 mL/min was found to be a superior eluant and is now routinely used. For the air quality study, separations were achieved on a 150 × 4.6 mm stainless steel column packed with Ultramex 3 Phenyl (Phenomenex, Torrance, CA) with acetonitrile/water (55:45) elution at 0.7 mL/min. Ergosterol was detected with a Varian 2550 variable wavelength detector (Varian Analytical Instruments, San Fernando, CA) set at 282 nm. Estimations of ergosterol were made by a comparison of peak areas with those of external standards. Confirmation of ergosterol was accomplished by a comparison of retention times with the external standard or by coinjection with a standard.

Gas Chromatographic−Mass Spectrometric (GC−MS) Analysis. Confirmation of ergosterol was also accomplished by using a Finnigan 4500 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) operating in the electron ionization (EI) mode at 70 eV. Quantitation was achieved by using multiple ion monitoring (*m/z* 337, 363, 396) with pure ergosterol as an external standard. A J&W Scientific DB5-MS 15 m × 0.25 mm i.d. column with 0.26 μm film (J&W Scientific, Folsom, CA) was used with on-column injection, and

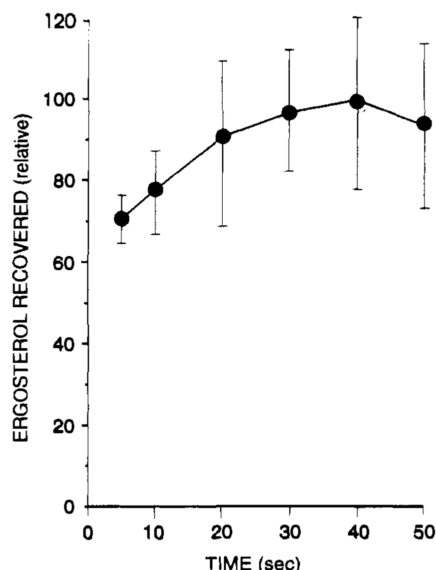

Figure 1. Effect of irradiation time on microwave-assisted extraction of ergosterol from *C. cladosporioides* spores. Values represent averages of triplicate determinations.

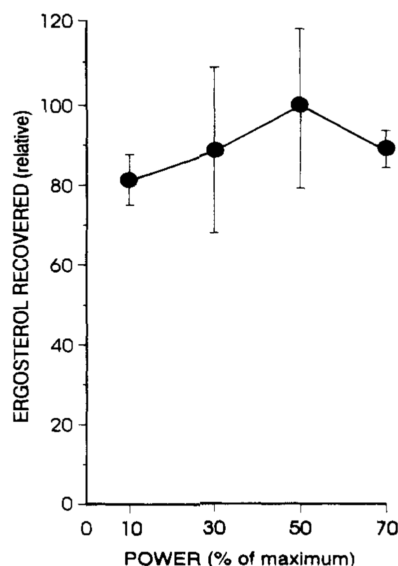

Figure 2. Effect of power on microwave-assisted extraction of ergosterol from *C. cladosporioides* spores. Values represent averages of triplicate determinations.

the column was temperature programmed from 150 to 300 °C at 20 °C/min.

Fatty acid profiles were determined on the same instrument fitted with a longer DB5 column (25 m) and temperature programmed from 120 to 300 °C at 20 °C/min. Fatty acids were identified by comparison of EI spectra with those in the data system library and congruence of chromatographic retention times with those of standards.

Determination of Spore Dimensions. The dried spores were suspended in distilled water and mounted on standard glass microscope slides with cover slips. The water mounts were sealed with clear nail lacquer. Images for 100 spores were collected using a Dage MTI black and white television camera at 512 × 512 pixel spatial resolution and 8 bits of gray depth from a Zeiss Universal light microscope (Carl Zeiss Canada Ltd., Don Mills, ON) using a 40× Neofluor objective. Image analysis on a Kontron Ibas image analysis system (Kontron Elektronic GmbH, Eiching, Germany) provided maximum and minimum dimensions for each spore.

RESULTS AND DISCUSSION

Microwave-Assisted Extraction of Ergosterol. From the outset, it was apparent that the concurrent extraction and saponification proceeded rapidly under microwaving conditions. The effects of various operating parameters (irradiation time, power, alcohol, base and concentration, and organic to aqueous solvent ratio) on the extent of extraction were examined in some detail with *Cladosporium cladosporioides* spores to determine the optimum conditions.

Figure 1 shows that even after only 5 s of irradiation about 70% of the ergosterol was extracted, with the maximum being achieved at about 30−40 s. Since the reactions were not immediately thermally quenched prior to workup, it is possible that some of the overall extraction occurred during the subsequent cool-down period.

Initial studies at full power and/or longer irradiation times resulted in some tubes leaking material, so lower power settings were used to avoid this problem. Figure 2 shows that even at minimum power settings (10%) considerable extraction had occurred by 30 s; 50% power was sufficient to obtain maximum recovery. Since the power setting was not absolutely crucial to the procedure, the actual oven energy output was not determined.

The outcome was independent of the alcohol chosen. Methanol, ethanol, 1-propanol, and 2-propanol gave essentially the same extraction efficiency. Methanol was ultimately chosen for the procedure because it did not partition into the pentane during the final extraction. A ratio of organic to aqueous solvent of about 4:1 gave the best results, with somewhat lower values being achieved when either higher or lower ratios were used. While the final temperatures of the reaction solutions immediately following irradiation were not determined, initial investigations qualitatively showed that the final temperature was directly proportional to the amount of base present in methanol/water mixtures. Tubes containing solvent only became warm to the touch, whereas those also containing alkali became too hot to handle without gloves. Sodium hydroxide gave a slightly higher extraction/saponification efficiency than either lithium or potassium hydroxide. Over a concentration range of 0.5−4 M sodium hydroxide, the highest yields were obtained at 2 M.

When the caps normally supplied with the culture tubes were used, some substances present in the liner material were also extracted and presented an interference in the HPLC determination step. This problem was overcome by substituting Teflon-lined caps.

Explosion of reaction vessels due to the rapid increase in temperature and pressure under microwaving conditions has been reported (Bose et al., 1991). Since the simpler use of an open reaction flask with a funnel as a loose top, as suggested by Bose et al. (1991), resulted in loss of solvents and solutes under our conditions, sealed culture tubes were used instead. As an added measure of safety, the culture tubes were enclosed inside sealed plastic bottles. In over 1000 analyses with small sample sizes, there were only three instances of a culture tube rupturing while under microwaving conditions; in all cases the outer plastic bottle prevented escape of any material or broken glass. The outer bottle precaution had an additional benefit: the insulating properties of the air inside kept the reaction from cooling too quickly and allowed for continued extraction/saponification at the elevated temperatures. Others have overcome the explosion problem with special Teflon-lined reaction bombs (Provan et al., 1994; Lopez-Avila et al., 1994).

In the air quality study, the filters had to be folded to be placed into the reaction tube. While being microwaved, the filters decomposed and enabled reagent to have full access to the fungal material. The resulting alkaline milky solution became clear upon acidification, and this fortuitously served as an internal indicator of neutrality.

The total volume of reagents relative to the size of the reaction vessel was found to be important. In the early stages of development of this procedure, 2.5 mL of reagent was treated in a 13 mL culture tube. When larger reagent volumes were used in this tube, leaking of material became a problem, probably due to insufficient headspace to accommodate the vapor produced by the high temperature and pressure. Use of larger tubes (17 mL) was also required for some samples with high organic content (e.g. corn silk samples of 20 mg or more). As noted above, in some subsequent applications of this method involving larger sample sizes (e.g. 250 mg of soil), tube rupture was overcome by microwaving in even larger 27 mL culture tubes. Subsequently, the largest tubes were used exclusively. Thus, when the procedure is scaled up for increased sample sizes and/or volumes, larger tubes should be employed. In several instances, conducting the irradiations in two stages (for 20 s and then, after several minutes, an additional 15 s) was found to be necessary.

The MAE procedure reported herein, relative to nonmicrowave techniques available, requires smaller samples and smaller reagent volumes (less than 10 mL of methanol, base, and pentane) and therefore is more economical with respect to reagent purchase and disposal costs; it is not labor intensive, is more rapid, uses conventional equipment (including a domestic, rather than specialized, microwave oven), and, other than the danger of tube rupture, is less hazardous. It should be noted that the use of such small sample sizes requires that special attention be paid to ensuring that samples be homogeneous and representative of the whole. As can be seen from the data in Table 1, MAE also consistently gives equal or higher results and the best precision (at about 4% RSD), relative to classical extraction or SFE, for both pure fungi and samples naturally contaminated by fungi. The significance of this and further data is discussed below.

Classical Extractions. The data in Table 1 show that classical extraction, in which extraction and saponification are conducted sequentially, gives equal or substantially lower recoveries of ergosterol when compared with the MAE procedure. Other researchers have compared sequential and simultaneous classical extractions; some (Schwadorf and Mueller, 1989; Zill et al., 1988; Padgett and Posey, 1993) have observed higher yields of ergosterol from that conducted simultaneously, while others (Newell et al., 1988) have reported reduced yields. The classical method of extraction typically requires larger samples and larger volumes of reagents, is labor intensive, and takes longer (a trained technician can conduct about 6–8 times more MAE extractions per day).

Supercritical Fluid Extractions. An earlier study (Young and Games, 1993) demonstrated the utility of SFE for the determination of relative fungal contamination on the basis of free ergosterol levels. In this study, the results from SFE were substantially lower than those from MAE (see Table 1). The difference can be partly attributed to MAE giving total ergosterol. It may also be due to the disparity in the abilities of the

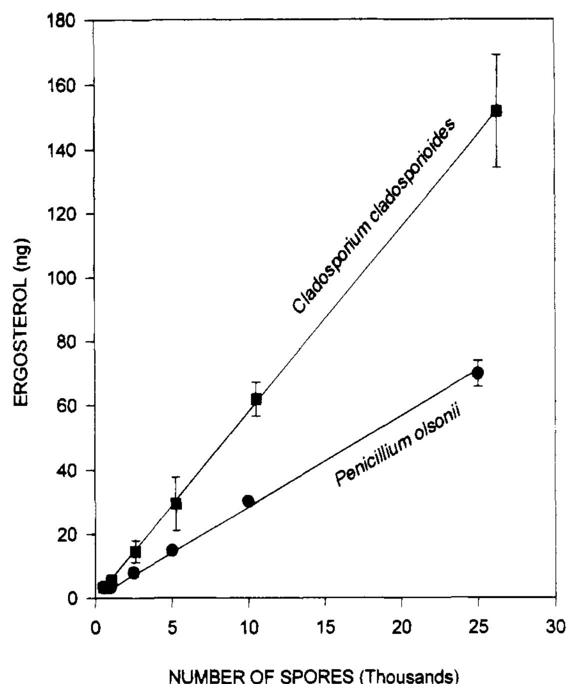

Figure 3. Ergosterol levels in *Cladosporium* and *Penicillium* spores as determined by microwave-assisted extraction and high-performance liquid chromatography with ultraviolet detection.

extracting solvents to perturb and penetrate the cell wall membrane and then remove ergosterol; recovery of ergosterol from inert material (e.g. filter paper) by SFE is virtually quantitative. Hot alcoholic alkali is presumably better at disrupting the membrane. Addition of a modifier (methanol) to supercritical CO_2 afforded higher extraction yields, although one was limited as to how much modifier could be added. In the instrument used, the effluent was trapped on a mini-ODS column and was later eluted with pure methanol; addition of too much methanol to the sample in the extraction thimble could have resulted in some ergosterol being prematurely stripped off the trap during the extraction step. Another difference may be related to the quality of carbon dioxide used. Supercritical fluid grade was used in this study, whereas the earlier study conducted in the United Kingdom used a "regular" grade without any special purification.

High-Performance Liquid Chromatography. In the past, elution of ODS columns with methanol or methanol/water (95:5) has provided sufficient resolution of ergosterol from impurities for quantitation purposes. However, some coextractives from filters used in the air quality study interfered with the ergosterol peak, and other chromatographic conditions had to be found. In that particular instance, use of an Ultramex 3 Phenyl column and elution with acetonitrile/water (55:45) provided the required resolution. In the soil application, the ODS/methanol combination was also subject to interference, and other eluants were investigated. Acetonitrile/methanol (80:20) achieved the desired separations; this combination has resulted in chromatography superior (especially narrower peak profiles) to that obtained with other eluants and has become the eluant of choice.

Gas Chromatography–Mass Spectrometry. In virtually all of the various ergosterol extraction procedures described in the literature, the reaction mixture after saponification was extracted directly without prior neutralization. It has been our experience that such

Table 3. Relative Levels of Fatty Acids and Their Methyl Esters in *A. alternata* Hyphae As Determined by Microwave-Assisted, Methanol, and Supercritical Fluid Extractions followed by Gas Chromatography–Mass Spectrometry

fatty acid	MAE[a]	classical[b]	SFE[c]
free acid			
C16:0[d]	29.3[e]	0.6	1.0
C18:0	5.4	nd[f]	nd
C18:1	9.8	nd	nd
C18:2	53.7	1.3	2.2
methyl ester			
C16:0	0.6	22.8	24.9
C18:0	nd	7.3	13.4
C18:1	0.6	15.0	29.2
C18:2	0.7	53.0	29.3

[a] Microwave-assisted extraction: samples were treated with 2 mL of methanol and 0.5 mL of aqueous 2 M sodium hydroxide and microwaved for 35 s at 50% power, then neutralized and extracted with pentane. [b] Classical extraction for ergosterol: samples were blended in methanol, centrifuged, refluxed for 30 min in ethanolic potassium hydroxide, and extracted with pentane. [c] Carbon dioxide supercritical fluid extraction: for 10 min at a density of 0.90 g/mL. [d] Carbon number and degree of unsaturation for straight-chain fatty acids and methyl esters. [e] Percent of total as determined by GC–MS. [f] Not detected.

extracts cause rapid degradation of the GC column (sometimes after only a few injections on-column). This also occurred when the MAE reaction mixture was extracted directly. Neutralization prior to pentane extraction overcame this problem. It was still found to be necessary to occasionally (every 20 injections or so) to remove about 1 m of the head of the column.

Limits of Detection. The detection limit of ergosterol for the HPLC-UV system used in this study was about 500 pg. Typically, 25 μL (of a 100 μL solution) was injected; thus, 2 ng/total sample would be the method detection limit. With GC–MS in the multiple ion detection mode, one could reduce this detection level by at least 100-fold. On the basis of results from the air quality study, by scanning only the ions at *m/z* 337, 363, and 396, the presence of 4.2 pg of ergosterol could be fully confirmed (a peak with the appropriate retention time and a spectrum with the three ions in the correct ratios). A 2 μL injection (of 100 μL) resulted in a method detection limit of 200 pg/total sample. By using only *m/z* 363, the instrument detection limit of 0.44 pg corresponded to a method detection limit of, for example, about 20 pg/m^3 for a 1 m^3 air sample.

Applications of MAE. *Ergosterol.* Ergosterol levels in several fungi in either the hyphal or spore stages are shown in Table 1. The levels for *Agaricus* caps and *Alternaria* hyphae are within the general range (about 0.1–15 mg/g of dry weight) reported for a wide variety of fungi (Newell et al., 1987; Newell, 1992; Gessner and Chauvet, 1993). Weete (1980a) observed that different strains of the same species grown under identical conditions can show considerable variation in lipid content. Thus, it is not surprising that the SFE value for ergosterol in the North American *Agaricus* mushroom caps used in this study (395 μg/g) would be different from that observed (14.3 mg/g) for caps obtained in the United Kingdom in the previous study (Young and Games, 1993). The utility of MAE to solid samples of mold-contaminated bread and corn was demonstrated by the good agreement with extractions by the classical method (Table 1).

One valuable application of MAE is the ability to determine ergosterol levels in very small samples. Although the spore data in Table 1 came from HPLC analysis of 5000–25 000 spores of each species, the levels per spore were linear to much smaller numbers, as illustrated in Figure 3.

Since ergosterol is a component of the cell membrane (Newell, 1992), the differences in ergosterol levels for the species reported can be attributed to their sizes. On the basis of the assumption of a prolate spheroid shape for each of the spores, and the determination of maximum and minimum dimensions for 100 representative spores for each species, the average volume and surface area for each were calculated. These values were then combined with the observed ergosterol levels to determine the relative ergosterol volume and surface area densities (Table 2). Although the volume densities in the species chosen were very different, there was good agreement for the amount of ergosterol per unit surface area, which ranged from 109 to 141 pg/μm^2. These results are consistent with the association of ergosterol with cell membranes.

In another study, corn silks were inoculated with *Fusarium graminearum* and/or *F. moniliforme* (Reid et al., 1992) and the progress of the disease was monitored by a variety of means, including MAE for ergosterol. Significant increases in ergosterol levels in the treated silks, compared with control, were detected within a few days of treatment (e.g. 9.6 vs 0.3 μg/g, respectively, after 6 days).

The presence of fungal spores is among the various factors that influence the quality of indoor air (Miller, 1995). As part of a study to evaluate the quality of air in Canadian homes, airborne fungal spores were filtered from air in two rooms (a bedroom and the largest living room) in each of 400 homes. The filters were then extracted by MAE and analyzed by HPLC-UV and GC–MS. These filters decomposed during the microwaving process. Because a component from extraction of the filter coeluted with ergosterol on the ODS HPLC column, a phenyl column was employed. Ergosterol was

Table 4. Levels of Total Fatty Acids in Spores of Various Fungi As Determined by Microwave-Assisted Extraction[a]

species	C8:0[b] (%)	C10:0 (%)	C12:0 (%)	C14:0 (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)
Aspergillus niger	0.1	1.4	58.2	28.3	8.5	2.5	1.0
A. ochraceus	0.6	3.8	56.0	26.4	10.1	2.3	0.8
A. sydowii	0.1	2.4	61.3	24.3	9.3	2.1	0.5
A. ustus	0.7	3.8	60.9	22.8	9.7	1.6	0.5
A. versicolor	0.2	2.6	55.9	26.7	10.6	2.9	1.0
Eurotium herbariorum	1.1	3.7	60.5	25.3	7.3	1.7	0.4
Penicillium brevicompactum	0.4	3.5	70.5	20.6	3.8	0.8	0.3
P. chrysogenum	1.8	3.9	58.9	25.1	7.1	2.4	0.8
P. commune	0	1.2	57.5	29.1	8.9	2.3	0.9
P. viridicatum	0.7	3.4	59.6	27.6	6.8	1.4	0.4

[a] Microwave-assisted extraction: samples were treated with 2 mL of methanol and 0.5 mL of aqueous 2 M sodium hydroxide and microwaved for 35 s at 50% power, then neutralized and extracted with pentane. [b] Fatty acids determined by GC–MS. Values are averages of quintuplicate extractions of about 20 000 spores.

detected in amounts ranging from 0 to 194 ng/m^3 of air (Miller and Young, 1995).

In the classical method for soil, about 8 g samples are used (Davis and Lamar, 1992; Scheu and Parkinson, 1994). With MAE, 250 mg samples were found to provide sufficient material for detection. A few representative samples, representing cultivated sandy, loam, and clay soils, as well as native forest and grassland soils were examined according to the MAE method. The cultivated soils showed lower ergosterol levels (1.15, 0.85, and 0.69 μg/g, respectively) than the undisturbed soils (2.21 and 3.10 μg/g, respectively).

For workers in grain elevators, there is concern over the potential risk of exposure to mycotoxins produced by fungi in grain dust (de Mers, 1994). Thus, the application of MAE to grain dust from a variety of Canadian elevators and grains was also evaluated. Observed ergosterol levels ranged from 3 to 327 μg/g (median 41 μg/g for 27 samples).

The full results of the corn silk, air quality, soil, and grain dust studies will be published elsewhere.

Fatty Acids. Partly on the basis of the utility of SFE for the analysis of fatty acids in *Agaricus* spp. mushrooms (Ibrahim Abdullah et al., 1994), the application of MAE to the analysis of FA in fungi was also investigated. Extracts used for the analysis of ergosterol were employed directly for the GC–MS analysis of FA. Table 3 compares the results of some fatty profile analyses by MAE, SFE, and classical techniques in *A. alternata* hyphae. The acids were observed as their methyl esters when extracted by the latter two techniques, whereas the hot alkaline conditions of MAE resulted in saponification of all fatty acid esters into their free acid form. The proportions of the various fatty acids (free or esterified) were similar from each technique.

Table 4 shows that for the dozen species of fungal spores studied, all showed very similar FA profiles. Others have also noted no major differences in FA profiles between related species (Sanchole and Dalpé, 1993). In this study, the C_{12}, C_{14}, and C_{16} saturated straight-chain acids predominated, with only minimal amounts of C_{18} saturated and monounsaturated acids observed (Table 4). In other studies on fungal spores (Weete, 1980b; Blomquist et al., 1992), the major acids reported were C18:2, C18:1, and C16:0, with C_{14} and shorter acids absent or observed only at trace levels. The disparity may be due to differences in the methods of extraction. The classical fatty acid method begins with a relatively mild total lipid extraction with chloroform/methanol at room temperature followed by saponification to release conjugated FA. The high-temperature and -pressure MAE procedure is much more vigorous and would result in the destruction of the organic structure of the spore or hyphae. The subsequent hydrolysis could release conjugated fatty acids not extracted by the classical method.

Summary. Microwave-assisted technology has been shown to be an efficient means for extracting the fungal metabolite ergosterol from spores, hyphae, and samples contaminated by fungi. Compared with the classical solvent extraction methods, the MAE procedure employed provides equal or higher extraction efficiencies, requires smaller samples and reagent volumes, is quicker (about 8-fold), uses "one pot", and is less labor intensive. For pure samples of spores or hyphae, the same extracts can also be used for the determination of total fatty acid profiles.

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