Determination of Phytosterols in Dried Shaggy Mane and Morel Mushrooms by GC-MS

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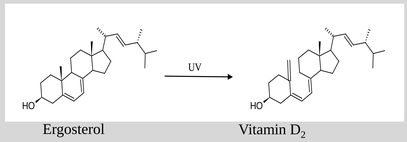
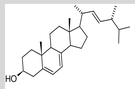
Background

Mushrooms are a very popular in many cultures, and have great nutritional value. For example the phytosterol, erogosterol is widely present in mushrooms, and when irradiated by UV light, it is converted into vitamin D_2 (eq1). [1,2] The purpose of vitamin D is to provide a healthy immune system, bone and muscle growth, and good cardiovascular health. People who are deficient in vitamin D, may be at risk for getting cardiovascular disease and cancer. Other phytosterols present in mushroom specie have been found to have relationship to lowering cholesterol. [4]

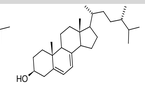


Ergosterol Vitamin D_2 (1)

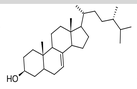
Phytosterols previously reported in edible mushrooms in mushrooms include ergosterol, ergosta-5,7-dienol, ergosta-7-enol, ergosta-7,22-dienol, brassicasterol, campesterol (compounds **1 - 6**).



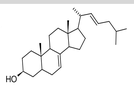
(1) Ergosta-5,7,22-trienol Ergosterol



(2) Ergosta-5,7-dienol



(3) Ergosta-7-enol Fungisterol



(4) Ergosta-7,22-dienol

(5) Ergosta-5,22-dienol Brassicasterol

(6) Ergosta-5-enol Campesterol

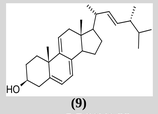
(7) Ergosta-5,24(28)-dienol 24-Methylenecholesterol

(8) Ergosta-7,24(28)-dienol Episterol

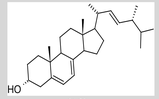
Previous research by others has analyzed edible fresh mushrooms collected in the field and freeze dried in the lab. Our research focused on analyzing store bought, edible, dried mushrooms. This was done by extracting the dried, ground mushrooms and analyzing the TMS-derivatized extracts by gas chromatography and mass spectrometry (GC-MS). In order to determine the absolute abundance of ergosterol in each species, an internal standard, cholesteryl stearate was added to the extracts. The mushrooms that were studied in this research were *Coprinus comatus* (shaggy mane) and *morchella* (morel), and *Ganoderma lucidium (*reishi). Shaggy manes are commonly found in Minnesota and other parts of North America. They are edible, but are not highly valued for people. This could be the reason why there has not been any literature reporting their phytosterol composition. Therefore, I was interested in analyzing their sterol content. Unlike shaggy manes, morels are a widely considered a delicacy in many cultures. There have been many studies on morel phytosterol composition, but there have not been studies comparing the phytosterol composition of morels with different ages. Therefore, we studied small (popcorn) versus large (jumbo) specimens of morels with the assumption that the small morels were picked much earlier in the fruiting stage.[6] However, the size can also be affected by forest disturbances, and other environmental factors.[6]

Results

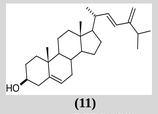
Previous work in our lab:
Store bought dried oyster, morel, shitake, and porcini mushrooms were analyzed and were found to have relative sterol concentrations and types that were closely related to those reported in fresh mushrooms.[1] Previously unidentified sterols found in dried oyster and morel mushrooms were able to be identified based on their mass spectra as sterols **7**, **8**, **9**, **10**, and **11** (tentative**)**. Another sterol, **12** has not yet been identified.



(9) ergosta-5,7,9(11),22-tetraenol



(10) epiergosterol



(11) ergosta-5,22,24(28)-trienol

Figure 1: TIC chromatogram of shaggy mane mushroom

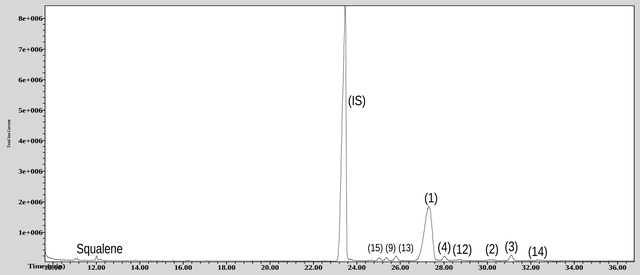


Figure 2: TIC chromatogram of popcorn size morel mushroom (Entry 3) Table 1)

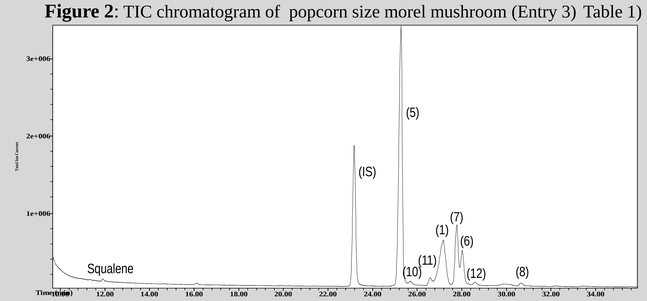


Figure 3: TIC chromatogram of popcorn morels upon reinjection (Entry 3[c] Table 1)

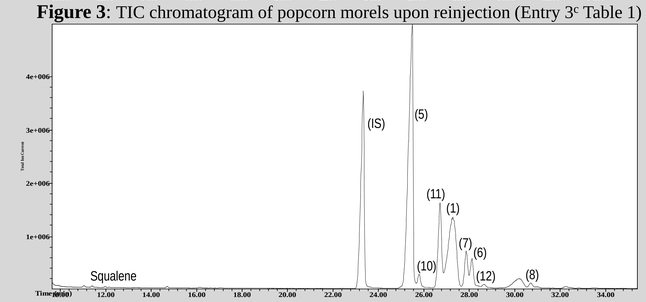
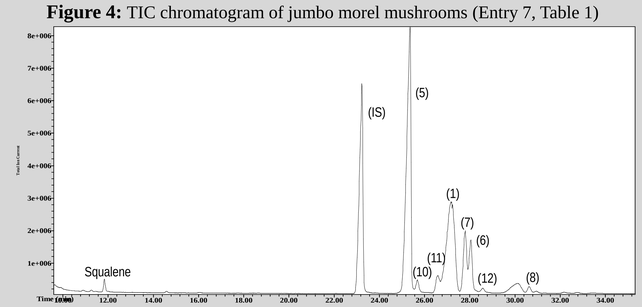


Figure 4: TIC chromatogram of jumbo morel mushrooms (Entry 7, Table 1)



Results

Shaggy Mane Mushroom

Morel Mushroom

Table 1: Relative Percent of Sterols in Dried Morel Mushrooms

Entry	Sample	Avg. Mass (g)	[1][a]	[1][b]	1	5	6	7	8	10	11	12
1	Popcorn	0.57	4.2	23.4	27.0	44.6	7.2	14.4	1.7	1.9	2.2	1.0
2	Popcorn	0.57	2.1	12.0	15.2	54.3	6.1	12.4	1.8	2.2	6.3	1.7
3	Popcorn	0.57	1.7	9.3	19.7	54.8	8.3	12.6	1.2	1.6	0.1	1.7
3[c]	Popcorn	0.57	1.9	10.7	24.3	50.6	3.7	5.1	1.1	2.0	12.4	0.8
4	Popcorn	0.57	3.3	18.6	24.8	49.2	7.6	10.7	1.5	1.7	2.8	1.7
5	Popcorn	0.57	3.0	16.6	25.5	50.9	3.8	14.4	2.0	1.7	0.9	0.8
Average		**0.57**	**2.9**	**16.0**	**22.4**	**50.8**	**6.6**	**12.9**	**1.6**	**1.8**	**2.5**	**1.4**
6	Jumbo	2.7	4.4	24.8	26.1	44.5	7.5	12.7	2.5	2.2	1.7	2.8
7	Jumbo	2.7	2.4	13.3	31.0	45.5	6.9	8.9	1.0	2.0	3.4	1.3
7[c]	Jumbo	2.7	1.5	8.6	22.3	52.8	7.8	9.1	1.2	1.9	3.1	1.8
8	Jumbo	2.7	3.6	20.5	30.0	38.0	8.4	8.6	4.1	2.7	5.5	2.7
9	Jumbo	2.6	4.0	22.3	24.1	48.4	5.2	17.2	0.9	1.7	1.6	0.9
10	Jumbo	3.3	1.7	9.5	24.6	49.0	6.4	14.5	2.6	1.8	0.0	1.1
Average		**2.8**	**3.2**	**18.1**	**27.2**	**45.1**	**6.9**	**12.4**	**2.2**	**2.1**	**2.4**	**1.8**
11	Regular[d]	-	-	-	24.9	51.0	3.9	4.2	1.5	1.2	13.4	0.0
12	Regular[d]	-	-	-	27.6	50.4	5.5	4.0	1.0	0.9	8.3	0.6
13	Regular[d]	-	-	-	22.9	54.6	5.7	5.8	1.5	1.2	7.8	0.6
14	Sundried[d]	-	-	-	32.7	43.8	5.8	7.5	1.4	0.7	7.1	0.7
15	Sundried[d]	-	-	-	25.5	46.9	5.4	7.8	1.3	0.8	11.2	0.6
16	Sundried[d]	-	-	-	21.8	55.6	6.6	9.7	0.5	0.6	5.1	0.0
Average		-	-	-	**25.9**	**50.4**	**5.5**	**6.5**	**1.2**	**0.9**	**8.8**	**0.4**
Total Average		**1.7**	**3.0**	**17.0**	**25.2**	**48.8**	**6.3**	**10.3**	**1.7**	**1.6**	**4.8**	**1.1**

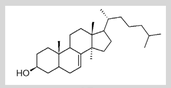
[a] mg ergosterol/100 g fresh weight, [b] mg ergosterol/100 g dry weight, [c] second injection (2-4 weeks after first injection), [d] Previously reported results from our lab

* [c] entries are not included in averages

Discussion

Early on in my research, reishi mushrooms, which are used for many medical purposes were studied. These mushrooms were found to be very complex in regards to their sterol composition, and there is ample literature reporting about their composition, so we decided not to report these results, and abandoned this line of research.

Shaggy mane phytosterol composition has not been reported in the literature. We found these mushrooms to contain a high relative abundance of ergosterol **(1)** (68-75%), however, the absolute mass of ergosterol wasn't particularly high **(**11-14 mg ergosterol/100 g). Sterols, **2**, **3**, **4**, and **9** were detected at trace levels. Thus, shaggy manes are unordinary in terms of their sterol composition. However, we did also detect the unexpected sterols, desmosterol **13**, lathosterol **14**, and lanosterol **15**.[10] (Figure 1). The % abundance of **14** in one of the samples was higher than in another entry (10.9/2.3 %). These compounds lack the usual methyl group attached to C-24 of the side chain and are not normally present in fungi. The presence of these sterols could suggest contamination by insect parts or other foreign material in this specific collection of dried shaggy manes. It is also possible that they are present as impurities in the cholesterol used as an internal standard.



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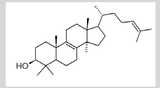

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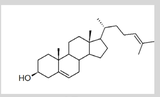

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A previously unidentified peak at about 12 min with M+ at *m/z* = 410 found in trace amounts in most samples was determined to be squalene. This was confirmed by comparing the mass spectrum to the literature. Squalene is a precursor in the biosynthesis of all phytosterols and so could have been expected to be present.[9]

Morels have a much more distinctive and varied composition of phytosterols and also contain more ergosterol than shaggy manes (Table 1). In accord with previous reports, brassicasterol **(5)** was found to be the most abundant sterol in morels. Small morels appeared to have a slightly higher % abundance of **5** than the jumbos (51% vs 45%) but both sizes had similar absolute masses of ergosterol (16 vs 18 mg/100g). Sterols **6** and **7** were the 3rd and 4th most abundant sterols. Overall, no major differences based on specimen size are evident in the data obtained (Table 1).

The change in sterol composition was also analyzed in two derivatized extracts during storage over a 2-4 week period. The results show sterols, **1** and **5** changed by more than 4% at the time of the second injection (Entries 3 and 3[c], 7 and 7[c]). For the popcorn size sample (entry 3), there was a major difference in the relative abundance of **11** with the initial injection showing a 0.1% abundance and the second injection showing 12.4%. Curiously, in the jumbo size (entry 7), **11** appeared to be stable over time (Table 1). Clearly, it is best to inject these samples with minimal storage time after sample preparation.

Experimental

Dried shaggy mane (Havista) and morel (multiple ebay sellers) were ground using an electric grinder (4-5 g sample). Cholesteryl stearate (~0.010 g, or 1.0 mL 5 mg/mL, Sigma Aldrich) was added to the mushroom sample, which was then Soxhlet extracted for 4 h with 150 mL of petroleum ether. The solvent was evaporated off at 60°C with a rotary evaporator. The extracts were saponified with 1 M NaOH/EtOH (4 mL) at 60-80° C for 1 h. The sterols were extracted with 2 mL of petroleum ether and washed with 2 mL of brine, and then washed two more times with petroleum ether. The extracts were dried with Na_2SO_4 , which removed the solvent. Derivatization with 0.40 mL trimethylsilylimidazole (TSIM) in 1.5 mL pyridine was done for 1 h at 60-80°C. The solution (1 µL) was injected into the GC-MS (initial temperature (T) = 250°C, ramp = 0.5°C/min, final T = 265°C, hold time = 25 min, gas flow = 0.9 mL/min). The period between when the extraction was made and when it was first injected varied (1-7 days).

Acknowledgments

Thank you to the Department of Chemistry at Winona State University for providing the resources necessary to complete this research and also to WSU Student Senate for travel funds.

References

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