9-1-2015

GC-MS Determination of Phytosterol Concentrations in Dried Mushrooms

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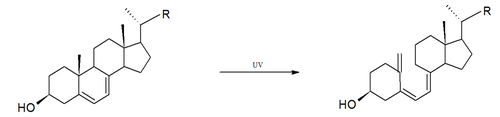
Background

Many types of edible mushrooms are found in the diets of different cultures worldwide. The composition and nutritional benefit of mushrooms is therefore of interest. Mushrooms are a known natural source of vitamin D, most notably vitamer D_2, which has been reported on extensively.[1,2] More recently, Vitamin D_4 has also been recognized for its presence and importance.[2] Vitamin D is well-known for its role in bone health and D_3 is produced in human skin during sun exposure. However, individuals with little to no sun exposure must obtain vitamin D from their diet. Vitamin D_2 and D_4 are produced from the respective sterol precursors ergosterol and ergosta-5,7-dienol when subjected to UV light (eq 1).[2]

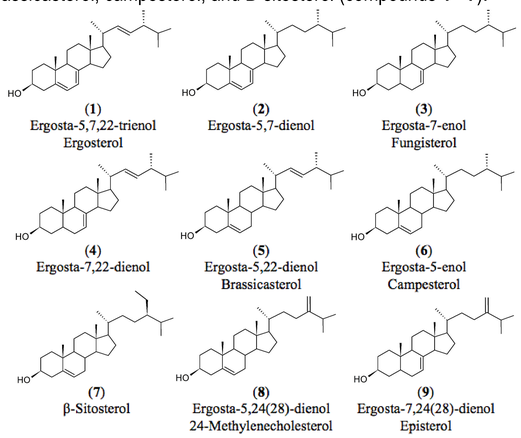


(1)

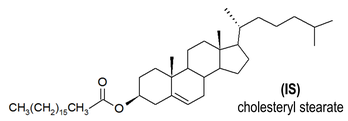
Ergosterol **(1)** / Ergosta-5,7-dienol **(2)** Vitamin D_2 / D_4

Along with providing a source of vitamin D, it is believed that dietary phytosterols may help lower LDL blood cholesterol due to their structural similarity with cholesterol.[4] In one study, rats were fed a mixture of phytosterols that decreased their overall cholesterol. Phytosterols are thought to lower cholesterol by directly inhibiting cholesterol absorption and displacing cholesterol in micelles.[4]

Phytosterols previously identified in mushrooms include ergosterol, ergosta-5,7-dienol, ergosta-7-enol, ergosta-7,22-dienol, brassicasterol, campesterol, and B-sitosterol (compounds **1** - **7**).



There have been many reports on the sterol content of edible, fresh mushrooms that are freeze-dried in lab. However, less work has been done on the sterol content of widely available, commercially dried mushrooms. Dried mushrooms have a better shelf-life, are easily reconstituted, and thought to be culinarily comparable to fresh. My research investigated the concentrations of sterols in various store bought dried mushrooms and compared them to that of fresh. Also the extraction time and methods were varied using cholesteryl stearate **(IS)** as an internal standard to determine optimum analytical conditions.

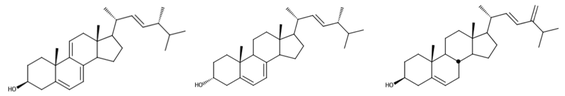


(IS) cholesteryl stearate

Results

Previous work in our lab

Store bought dried *Pleurotus ostreatus (*oyster), *Morchella* (morel), and *Boletus edulis* (porcini) mushrooms were investigated and found to contain relative sterol concentrations and types very similar to those reported in fresh mushrooms.[1] Previously unidentified sterols in dried oyster and morel mushrooms were identified based on their mass spectra as sterols **8**, **9**, **10**, **11**, and tentatively **12**.



(10) ergosta-5,7,9(11),22-tetraenol **(11)** epiergosterol **(12)** ergosta-5,22,24(28)-trienol

My research

Soxhlet extraction with petroleum ether was done using oyster mushrooms for varying times in order to determine the time required for maximum sterol extraction. In previous research no internal standard was used, leaving absolute amounts of sterols unknown. This research used cholesteryl stearate as an internal standard. Two different grinding methods, a mortar and pestle and an electric coffee bean grinder, were also investigated to the effect of grinding on sterol extraction. In addition, *Lactarius indigo* (blue indigo), *Tuber indicum* (black truffle), and *Lentinula edodes* (shiitake) were also analyzed for sterol concentration.

Figure 1: GC of oyster mushrooms with internal standard

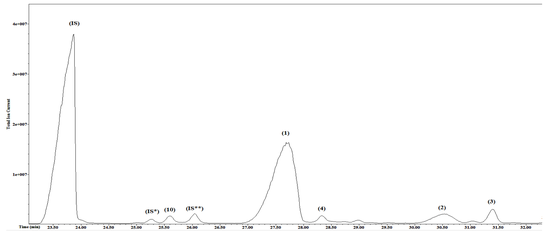
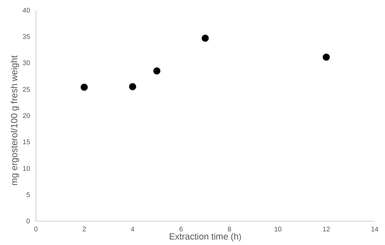


Table 1: Retention times of sterols found in mushrooms

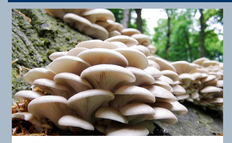
Sample	**10**	**5**	**11**	**1**	**6**	**4**	**2**	**3**
Oyster	25.6	-	26.1[a]	27.7	-	28.3	30.5	31.4
	(1.000)		(1.020)	(1.082)		(1.105)	(1.191)	(1.227)
Shiitake	25.6	-	-	28.0	-	28.5	30.6	31.6
	(1.000)			(1.094)		(1.113)	(1.195)	(1.234)
Truffle		25.6	-	-	28.3	-	-	31.3
		(1.000)		(1.078)	(1.105)			(1.223)
Blue Indigo	-	-	26.0[a]	27.5	-	28.2	-	31.3
			(1.016)	(1.074)		(1.117)		(1.223)
Lit.[b]	25.8	26.6	27.1	28.6	29.6	29.6	32.0	33.0
	(0.970)	(1.000)	(1.019)	(1.075)	(1.113)	(1.113)	(1.203)	(1.241)

[a] may have overlap with IS, [b]Reference 3, ( ) Relative retention indices based on **5**

Figure 2: Graph of ergosterol extracted vs. extraction time



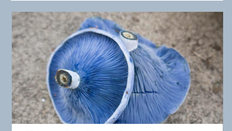
Results



Oyster Mushrooms

Black Truffle Mushrooms

Shiitake Mushrooms



Blue Indigo Mushrooms

Table 2: Relative percent of sterols found in dried and fresh mushrooms

Entry	Sample	Type	Extraction Time (h)	1		2	3	4	5	6	10	11
				[1][d]	[1][e]	Relative % Abundance of Sterols						
1	Oyster[a, f]	Dried	4	29.6	166	83.9	5.3	3.2	2.4	-	-	2.4	2.8
2	Oyster[b, f]	Dried	4	16.0	89.7	87.8	4.1	2.9	1.4	-	-	0.9	2.8
3	Oyster[c]	Fresh	-	68.0	-	83.3	10.5	2.5	2.3	-	-	-	0.8
4	Oyster[b, g]	Dried	2	25.4	142.7	82.2	8.8	3.6	1.6	-	-	1.1	2.7
5	Oyster[b, f]	Dried	4	16.2	91.3	86.0	4.8	3.2	2.4	-	-	0.9	2.7
6	Oyster[b, g]	Dried	5	28.5	160.1	77.3	7.8	4.7	2.3	-	-	2.5	5.4
7	Oyster[b, g]	Dried	7	34.7	195.0	78.9	6.3	4.2	2.4	-	-	3.5	4.8
8	Oyster[b, g]	Dried	12	31.1	174.9	79.4	9.7	3.6	1.9	-	-	1.9	3.5
9	Shiitake[a]	Dried	4	46.4	261	78.5	3.1	12.9	4.6	-	-	0.8	-
10	Shiitake[c]	Fresh	-	84.9	-	87.1	3.5	6.6	2.3	-	-	0.7	-
11	Morel[b]	Dried	4	-	-	25.1	-	-	-	52.0	5.0	-	-
12	Morel[c]	Fresh	-	26.3	-	34.0	4.0	1.9	-	41.6	4.6	-	1.2
13	Truffle[a]	Dried	4	12.6	70.6	51.1	-	2.1	-	42.4	4.3	-	-
14	Truffle[h]	Fresh	-	-	-	25.3	-	2.0	-	63.1	4.1	-	-
15	Blue In[b]	Dried	4	23.7	133	91.5	-	4.2	1.9	-	-	-	2.5

[a] ground to a powder, [b] ground coarsely using mortar and pestle, [c] Reference 3,
[d] mg ergosterol/100 g fresh weight, [e] mg ergosterol/100 g dry weight, [f] obtained from The Mushroom House, [g] obtained from Festival Foods [h] Reference 5

Discussion

Extraction of ergosterol relative to cholesterol was found to only slightly increase with extraction times greater than two hours (Figure 2), thus, the 4-h extraction time used for our lab's previous research was adequate.[6] Thoroughly pulverizing with a coffee grinder led to the extraction of 185% more ergosterol than using a mortar and pestle as done in our previous research (Table 2, Entries 1 and 2). However, the relative concentrations of the sterols extracted were relatively invariant, again lending confidence to our previous results.

Both dried oyster and shiitake mushrooms showed a statistically lower concentration and relative percent of **(1)** compared to that reported on in fresh mushrooms. Some uncertainty in our concentration of **(1)** comes from an estimated dry-to-fresh conversion ratio (17.8g:100g). Dried shiitake mushrooms showed a higher relative percent of **(3)** and **(4)** compared to the literature, while the oysters were more similar to the literature. Dried blue indigo mushrooms (entry 15) showed very similar sterol content to that of oysters.

For an unknown reason, dried truffles showed a higher concentration of **(1)** and a lower concentration **(5)** of compared to the literature fresh truffles (entries 13, 14). Interestingly, truffles and morels showed high concentrations of **(5)**, suggesting common biosynthetic processes between the two species.

Conclusions

- Grinding dried mushrooms to a powder produces a larger concentration of extracted sterols.
- Maximum sterol extraction is thought to be reached within 4 h for non-ground mushrooms.
- Commercially dried oyster and shiitake mushrooms showed similar relative percentages of sterols to fresh mushrooms from literature.
- Dried truffles showed a higher relative percent of ergosterol than that of the literature.

Experimental

Dried oyster (The Mushroom House), dried shiitake (Harmony House Foods, Inc.), dried black truffles (MarDona, China), and sundried blue indigo mushrooms (wild, harvested in Dakota, MN) were ground to a powder using either an electric grinder or a mortar and pestle (~4g sample). Cholesteryl stearate (~0.012 g, Sigma Aldrich) was added to the mushroom sample and Soxhlet extracted for 4-5 h with 150 mL of petroleum ether. The solvent was evaporated off at 60°C under vacuum. The extracts were saponified with 1 M NaOH (3 mL) at 60-80° C for 1 h. The sterols were extracted with 3 mL of petroleum ether and washed with 3 mL of brine three times each. The combined extracts were dried with Na_2SO_4 and the solvent was removed. Derivatization with 0.50 mL trimethylsilylimidazole (TSIM) in 1.0 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)

Acknowledgments

Special thanks to Todd Fuller for harvesting and drying the blue indigo mushrooms used.

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(7) Photograph : Oyster mushrooms, 2014 Fresh Crop Mushrooms.
(8) Photograph: Black truffle mushrooms, wikipedia.com (accessed Feb. 2016).
(9) Photograph: Shiitake mushrooms, Keith Weller
(10) Photograph: Blue indigo mushrooms, 2015 Tyrant Farms