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A New Analytical Fingerprinting Method for Quality Control of Medicinal Mushroom Products

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ABSTRACT

A systematic scientific testing methodology is proposed for medicinal mushroom products. Sixty samples representing 6 species of basidiomycetes and ascomycetes were evaluated for β-(1–3)(1–6)-glucans, α-glucans, and ergosterol. Using a fruit body for a baseline, test results demonstrate that β-glucans and ergosterol provide a value range that permits the development of a fingerprint and enables accurate quality control over a range of medicinal mushroom morphological stages. Testing for α-glucan reveals levels of starch-based adulterants and carrier materials.
Keywords: Medicinal Mushrooms, Mycelium, Alpha-Glucan, Beta-Glucan, Ergosterol

INTRODUCTION

Medicinal mushrooms (MM) is the common name given to certain basidiomycetes and ascomycetes marketed as health foods or dietary supplements. MM are part of an extensive range of health products in Asia where Chinese traditional medicine has utilized fungi and plant preparations for thousands of years. Today, different morphological stages of MM, some traditional and others more modern, are marketed in Asia and internationally. These stages include the fruit body (mushroom), sclerotium, mycelium, and spores. Mushrooms and spores are produced by standard industrial cultivation methods whereas mycelium is produced by fermentation or as a solid state grain spawn. Thousands of branded products made from the various fungal stages are sold worldwide, even though no systematic scientific verification of active or marker compounds has been established. Despite 50 years of research that has identified and characterized the primary medicinal constituents of MM, few pure analytical standards are available with which to measure or quantify them.

To provide a qualitative and quantitative measurement of known active compounds in a MM product, scientifically validated and commercially available methodologies for practical use in quality control were identified, in addition to a method for common product adulterants and carrier materials used in fungal extract products. The fruit body and sclerotium are utilized as a baseline for fingerprinting since those are the stages of MM historically utilized in traditional medicine and upon which most MM research is based. The fruit body is also the standard used for product identity. MM products were evaluated (Tables 1 & 2) on the basis of two primary active compounds of fruit bodies: β-(1–3)(1–6)-glucans and ergosterol. α-Glucan analysis is utilized as a means of identifying starches, which are common carrier materials. Most β-glucan testing methods are designed specifically for grains. The use of these grain-specific tests is not accurate for mushrooms. The presence of starch in a mushroom product can artificially elevate quantitative test results for

polysaccharides and give a false-positive value for the medicinal β-D-glucans. For that reason, it is important to utilize a test method specific to mushroom β-glucans which will also separate the α-glucans from the total.

Table 1. β-Glucan, α-Glucan, and Ergosterol Values of Chaga (*Inonotus obliquus*), *Cordyceps militaris*, *Cordyceps* Anamorphs, and *Trametes* (*T. versicolor*)

Sample	Description	β-D-Glucan	α-Glucan	Ergosterol
M1	Chaga sclerotia*	7.44	0.11	
M2	Chaga sclerotia*	6.79	1.05	0.040
ME1	Chaga sclerotia extract	11.95	1.11	
ME2	Chaga sclerotia extract	11.93	0.80	0.030
GS1	Chaga mycelium - rice	0.0	70.0	
GS2	Chaga mycelium - sorghum	4.18	28.22	0.007
GS3	Chaga mycelium - rice	7.36	44.14	0.000
GS4	Chaga mycelium - rice	6.20	40.17	
M3	*Cordyceps militaris* fruit body*	37.0	2.0	
M4	*C. militaris* fruit body*	34.4	1.7	
ME3	*C. militaris* fruit body extract	33.0	3.7	0.31**
GS5	*Cordyceps* mycelium - oats	1.0	45.1	
GS6	*Cordyceps* mycelium - sorghum	5.2	24.1	
GS7	*Cordyceps* mycelium - sorghum	10.9	53.9	
GS8	*Cordyceps* mycelium - sorghum	1.5	64.0	
GS9	*Cordyceps* mycelium - rice	2.3	46.3	0.006**
PM1	*Cordyceps* mycelium Cs-4	10.6	4.6	0.709**
PM2	*Cordyceps* mycelium Cs-4	7.85	1.71	0.030
M5	*Trametes* fruit body	57.3	0.1	
M6	*Trametes* fruit body	65.9	0.2	
ME4	*Trametes* fruit body extract	55.4	0.4	0.000
ME5	*Trametes* fruit body extract	55.8	0.2	
GS10	*Trametes* mycelium - sorghum	6.70	24.55	0.012
GS11	*Trametes* mycelium - rice	9.06	44.78	0.010
	Brown rice - cooked	0.85	73.67	0.000
	Sorghum grain - cooked	1.89	63.52	

Cordyceps mycelium products = uncharacterized anamorphs labeled as *Cordyceps sinensis*. Cs-4 is produced by fermentation. *Samples tested at SGS Laboratories and Megazyme International Ireland. **Analyses by BioProfile Laboratories. All data are expressed as % dry weight. Empty cell = not tested.

A method specifically designed for β-D-glucans in mushrooms and yeast was developed in 2004 by Megazyme International Ireland (Megazyme, 2016). The Megazyme method detects and quantifies soluble and insoluble β-(1–3)(1–6)-glucans. Using the Megazyme method, all stages of MM products can be tested, whether fruit bodies, fruit body extracts, mycelium, or even grain spawn mycelium. β-Glucan testing was performed by SGS Laboratories, Vancouver, B.C. A subset of sample results was validated and confirmed by the laboratory of Megazyme International Ireland.

Ergosterol, the major fungal sterol, is a definitive marker compound that can be used to assess the amount of fungal material in a given product (Dalla-Santa et al., 2012; Ng et al., 2008). Ergosterol testing was performed by Dr. Katherine Phillips, Virginia Tech, Blacksburg, VA. Dr. Phillips

conducts ongoing research on ergosterol and vitamin D2 (Simon et al., 2011). Ergosterol tests were also performed by BioProfile Laboratories, St. Paul, MN.

The presence of starch in dietary supplements, especially as a carrier for extract products, is an ongoing industry issue. MM grain spawn products present a similar issue, since the grain is not separated from the mycelium. Testing for α-glucans will determine the level of grain residue present in grain-based mycelium. It will also identify starch carriers used in mushroom extracts. Mushrooms contain only minor amounts of starch; on average, less than 3% dry weight (Kalač, 2013). The Megazyme β-glucan test kit also measures α-glucan (Megazyme, 2016).

Table 2. β-Glucan, α-Glucan, and Ergosterol Values of Maitake (*Grifola frondosa*), Reishi (*Ganoderma lucidum*), and Shiitake (*Lentinula edodes*)

Sample	Description	β-D-Glucan	α-Glucan	Ergosterol
M7	Maitake fruit body	36.7	1.2	
M8	Maitake fruit body*	36.0	1.8	
M9	Maitake fruit body*	40.58	2.02	0.490
M10	Maitake fruit body	46.2	5.7	0.391**
ME6	Maitake fruit body extract	37.95	5.23	0.510**
ME7	Maitake fruit body extract	40.80	3.33	0.330
GS12	Maitake mycelium - sorghum	3.46	39.91	0.007
GS13	Maitake mycelium - rice	6.38	44.47	0.032
M11	Reishi fruit body*	33.1	0.1	
M12	Reishi fruit body	29.8	0.7	
M13	Reishi fruit body*	31.9	0.2	
M14	Reishi fruit body	25.40	0.46	0.120
ME8	Reishi fruit body extract	43.47	0.66	0.090
ME9	Reishi fruit body extract	40.77	2.35	0.080**
GS14	Reishi mycelium - sorghum	5.96	29.61	0.019
GS15	Reishi mycelium - sorghum	10.01	31.51	0.006
GS16	Reishi mycelium - rice	4.48	48.88	0.006
GS17	Reishi mycelium - rice	9.76	49.13	
GS18	Reishi mycelium - sorghum	7.3	45.2	
GS19	Reishi mycelium - sorghum	12.9	31.5	
GS20	Reishi mycelium - oats	1.0	38.9	
GS21	Reishi mycelium - rice	1.1	51.4	
M15	Shiitake fruit body	35.39	3.46	0.230
M16	Shiitake fruit body	31.3	0.78	
M17	Shiitake fruit body	34.0	2.10	
M18	Shiitake fruit body	25.60	3.06	0.520
ME10	Shiitake fruit body extract	35.92	2.62	
ME11	Shiitake fruit body extract	30.41	3.77	0.150
GS22	Shiitake mycelium - sorghum	7.20	38.88	0.016

*Identical samples tested at SGS and Megazyme International. **Analyses by BioProfile Laboratories.
All data are expressed as % dry weight. Empty cell = not tested.

Samples of MM were selected from 6 species of fungi in 3 major groupings: fruit bodies, fruit body extracts, and grain spawn mycelium. Commercial mycelium products (grain spawn) manufactured in the U.S. were purchased from online resellers. In total, 22 samples were obtained from 13 companies. Two samples are pure *Cordyceps* mycelium from China. Dried mushrooms (n = 18)

were selected from retail packaged products from China and identified by the author. A second set of mushrooms was purchased fresh in the markets of Vancouver, B.C. by mycologist Paul Kroeger, who then dried them. *Trametes versicolor* was harvested wild in British Columbia. All mushroom extracts (n = 11) were manufactured in China for Nammex. Extracts were prepared by cooking coarsely ground mushrooms for 3 hours in hot water (80 °C), evaporating the fluid and drying it on the mushroom marc. The extraction ratio was 1:1 (1 kg dry raw material = 1 kg dried extract).

Results & Discussion

β-Glucan results show a consistent pattern in fruit bodies and fruit body extracts with a quantitative range of 25.4–65.9% (Tables 1 & 2). It has been reported that β-glucan levels can be affected by the specific cultivar and available nutrients of the substrate (Bak et al., 2015). This may account for some values outside of the range of results. β-Glucan in grain spawn mycelium products is significantly lower and consistently below 10%. This may reflect the dominance of grain, which would dilute the mycelial mass. In some grain spawn products, β-glucan is nearly absent.

Levels of α-glucan are low in the mushrooms and mushroom extracts, which is consistent with historical nutritional data showing little to no starch in mushrooms (Kalač, 2013). This is also an indication that a MM product has not been adulterated by starch. The grain spawn mycelium products contain α-glucan in the range of 24.1–70% (Tables 1 & 2). This provides further evidence that grain is the dominant component of these products and the likely reason for lower β-glucan levels compared to those of fruit bodies and fruit body extracts.

Ergosterol levels are highest in fruit bodies—especially the fleshy fungi—and lower in the polypores and chaga sclerotia. In general, the grain spawn mycelium products contain significantly less ergosterol (Tables 1 & 2). These results indicate that grain spawn mycelium products contain consistently low amounts of fungal tissue.

Using the three specific compounds and the test methods outlined in this study provides a primary means for a comprehensive fingerprint of MM products for the purpose of quality control. Additional compounds specific to individual species, such as triterpenoids in reishi and chaga and cordycepin in *Cordyceps militaris*, will expand and enrich the scope of this fingerprinting method.

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