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Chemical Composition of the Tiger's Milk Mushroom, *Lignosus rhinocerotis* (Cooke) Ryvarden, from Different Developmental Stages

Beng Fye Lau, Noorlidah Abdullah,* and Norhaniza Aminudin

Mushroom Research Centre, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Supporting Information

ABSTRACT: The chemical composition of the tiger's milk mushroom (*Lignosus rhinocerotis*) from different developmental stages, i.e., the fruit body, sclerotium, and mycelium, was investigated for the first time. The fruit body and sclerotium of *L. rhinocerotis* were rich in carbohydrates and dietary fibers but low in fat. Protein levels in *L. rhinocerotis* were moderate, and all essential amino acids, except tryptophan, were present. The mycelium contained high levels of potassium, phosphorus, magnesium, riboflavin, and niacin and appreciable amounts of essential fatty acids. The results indicated that the sclerotium of *L. rhinocerotis* that was used in ethnomedicine was not superior to the fruit body and mycelium with regard to the nutritional content and bioactive constituents. Our findings provide some insights into the selection of appropriate mushroom part(s) of *L. rhinocerotis* and proper cultivation techniques for the development of new nutraceuticals or dietary supplements.

KEYWORDS: *mushroom cultivation, sclerotium, mycelium, dietary fibers, nutraceuticals*

INTRODUCTION

Wild mushrooms collected by communities in many parts of the world are extensively used in cooking and cuisines. Some are also used in traditional medicine practices. *Lignosus rhinocerotis* (as '*rhinocerus*') (Cooke) Ryvarden is a polypore that thrives in tropical forests. It forms a large sclerotium that resembles a congealed mass of tiger's milk; hence, it is known as the "tiger's milk mushroom". Among the native communities, the sclerotium of *L. rhinocerotis* has a long history of use as a general tonic and natural remedy for ailments as well as to stave off hunger.[1]

Currently, the main supply of *L. rhinocerotis* is from the wild and its existence is often solitary. The increase in demand of this species has led to overharvesting, and the depletion of wild resource is concerning. In view of this, artificial cultivation of *L. rhinocerotis* was prioritized whereby fruit body and sclerotium were successfully induced from solid-substrate fermentation on agroresidues,[2] while liquid fermentation was optimized for the production of mycelium.[3] As a result, mushroom samples from both cultivation techniques, representing distinct fungal developmental/physiological stages, are available.

Nutritional and medicinal properties of mushrooms are often related to their chemical composition, but for *L. rhinocerotis*, this aspect has yet to be explored extensively. Limited studies are due to difficulties in domesticating the mushroom and/or obtaining large sample amounts from the wild. Literature on chemical composition of sclerotia-forming mushrooms, except for *Pleurotus tuber-regium*, is fairly scarce. Although there are numerous reports on the chemical composition of wild and edible mushrooms, very few had focused on comparative analyses of mushroom samples from different developmental stages.

From the ethnobotanical point of view, only the sclerotium of *L. rhinocerotis* has been claimed to have medicinal properties and to be good for overall wellness.[1] Previously, the proximate composition of *Polyporus rhinocerus* (synonym for *L. rhinocerotis*) from China has been reported, but only the sclerotium was analyzed.[4] From the nutritional aspect, the sclerotium of *L. rhinocerotis* is considered to be a good source of dietary fiber rich in β-glucans.[5] However, the potential of other mushroom parts was not investigated. In view of that, it is necessary to expand the present study to include the chemical composition of different parts of the fruit body, i.e., pileus and stipe, as well as the mycelium of *L. rhinocerotis*.

Comparison of the chemical composition and nutritional attributes of the pileus, stipe, sclerotium, and mycelium will be the main objective of this study. In the context of its chemical composition, the potential utilization of *L. rhinocerotis* fruit body and mycelium as substitute for the sclerotium as a new source of nutraceuticals and dietary supplements will be discussed.

MATERIALS AND METHODS

Mushroom Material. The fruit body (basidiocarp) and sclerotium of *L. rhinocerotis* were collected from Kenaboi Forest Reserve, Negeri Sembilan, Malaysia, and authenticated by mycologists from Mushroom Research Centre (MRC), University of Malaya. A voucher specimen was deposited at the University of Malaya herbarium, and axenic cultures were kept in MRC culture collections (KUM61075). Cultures were maintained by periodic subculturing on malt extract agar (MEA, Oxoid) at 4 °C.

Mushroom Cultivation. The fruit body and sclerotium of *L. rhinocerotis* were produced by solid-substrate fermentation of agroresidues,[2] while submerged fermentation was used for production of the mycelium. Mycelial plugs (diameter: 10 mm) used as inoculum were cut from the periphery of 13−15-day cultures growing on MEA plates incubated at 25 °C.

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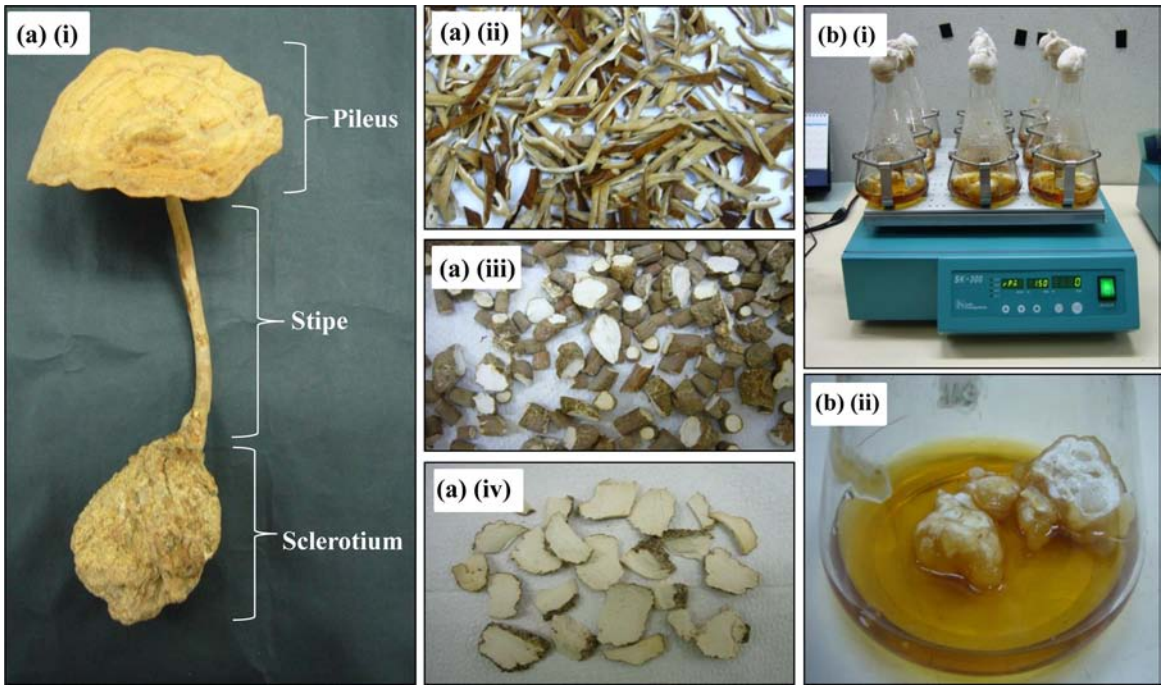

Figure 1. Samples of *L. rhinocerotis* from different developmental stages. (a) (i) The fruit body consists of pileus (cap) and stipe (stalk) attached to a sclerotium, which is buried underground. Sliced mushroom samples, (ii) pileus, (iii) stipe, and (iv) sclerotium, were dried in the oven. (b) (i) Submerged fermentation of *L. rhinocerotis* at room temperature. (ii) Growth in the form of mycelial pellets under shaking conditions.

Solid-Substrate Fermentation. Briefly, an optimized formulation of agroresidues consisting of sawdust, paddy straw, and spent yeast at pH 6.0 and 60% (v/w) moisture content was filled into transparent plastic polypropylene bags and sterilized. The substrate bags were inoculated with 10 mycelial plugs and incubated for approximately 60 days under dark conditions at room temperature (25 ± 2 °C). Once the substrate was fully colonized, the polypropylene bag was removed and the substrate block was buried for sclerotium development. Formation of fruit body and sclerotium was induced by continuous watering. Matured fruit body and sclerotium of *L. rhinocerotis,* as shown in Figure 1(a), were harvested approximately 12 months later. Pileus and stipe of the fruit body were separated. Mushroom samples were cleaned and placed in the oven at 40 °C until dried.

Submerged Fermentation. Basal fermentation medium consists of 1.5% (w/v) glucose (Merck), 0.8% (w/v) yeast extract (Oxoid), 0.8% (w/v) malt extract (Oxoid), and 0.8% (w/v) peptone (Oxoid) (GYMP) fortified with the following salts (analytical grade): 0.1% (w/v) KH_2PO_4, 0.1% (w/v) K_2HPO_4, 0.1% (w/v) NH_4Cl, and 0.1% (w/v) $MgSO_4 \cdot 7H_2O$. GYMP medium was prepared in 500 mL Erlenmeyer flasks, and the pH was adjusted to 6.0−6.5. The medium was sterilized and inoculated with 10 mycelial plugs per flask. Inoculated flasks were then placed on a rotary shaker at 150 rpm, as shown in Figure 1(b)(i). After incubation of 15 days at room temperature (25 ± 2 °C), submerged cultures of *L. rhinocerotis* were harvested. Mycelial pellets, as shown in Figure 1(b)(ii), were filtered off the culture broth, washed extensively with distilled water, and freeze-dried.

Chemical Composition Analysis. Samples of *L. rhinocerotis,* i.e., pileus, stipe, sclerotium, and mycelium, were cut into smaller pieces as shown in Figure 1(a)(ii−iv), ground in a Waring blender to pass a 40 mesh size, and kept at 4 °C prior to analysis. Chemical composition of mushroom samples was analyzed according to standard protocols by AOAC International, American Oil Chemists' Society (AOCS), and the American Association of Cereal Chemists (AACC) as described below. Each analysis was performed in triplicate. Results are presented as mean values and standard deviation. The data were analyzed statistically using the IBM SPSS Statistics Version 19 (SPSS Inc., an IBM company, USA). All mean values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-HSD ($p < 0.05$) to detect significance between groups.

Proximate Composition. Proximate composition of *L. rhinocerotis* was estimated according to standard protocols and expressed on a dry weight (DW) basis. Crude protein was determined using the Kjedahl method with boric acid modification (AACC 46-12), while crude fat was determined based on the milk method (AOAC 985.01). Total ash was measured using the basic method (AACC 08-01). Moisture content was determined by the air-oven method (AACC 44-15A). Fiber was analyzed according to AACC 32-10. Total carbohydrates and energy content were calculated by difference, whereby total carbohydrates (g) = 100 − (crude protein + crude fat + ash + moisture) and energy content (kcal/100 g) = [(4 × crude protein) + (9 × crude fat) + (4 × carbohydrates)]

Fatty Acid Composition. Fatty acid profiling of *L. rhinocerotis* was carried out by UNIPEQ Sdn. Bhd. (Bangi, Selangor, Malaysia). Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography (HP 5890 Series II Plus) equipped with a flame ionization detector (GC-FID) and a DB-205 column (30 mm × 0.25 mm × 0.25 mm). The oven temperature was initially set at 30 °C, then programmed to 250 °C using helium as the carrier gas at a flow rate of 1.3 mL/min and held for 30 min. Identification of fatty acids was done by comparing the retention time of FAME peaks with Supelco 37 FAME mixture standards (Sigma). Results were expressed as relative percentage of each fatty acid calculated by internal normalization of the chromatographic peak area.

Minerals and Vitamins Content. Concentration of the minerals (except selenium) in *L. rhinocerotis* was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer Optima 7300 DV) according to AOAC 985.01, AOAC 922.02, and AACC 40-70. For selenium, microwave-assisted digestion was used for sample preparation prior to analysis by inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer Elan 900). The levels of vitamin B complexes were assayed using high-performance liquid chromatography (HPLC) with a UV detector based on protocols described elsewhere.[6]

β-Glucans. The levels of 1:3- and 1:6-β-D-glucans in mushroom samples were measured using the mushroom and yeast β-glucan assay kit (Megazyme International Ireland) according to recommended

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Table 1. Proximate Composition (g/100 g DW) and Energy Values (kcal/100 g DW) of the Fruit Body, Sclerotium, and Mycelium of *L. rhinocerotis*[a]

	fruit body			
parameter	pileus	stipe	sclerotium	mycelium
carbohydrates	81.03 ± 0.13 a	80.09 ± 0.69 b	82.60 ± 0.01 c	73.01 ± 0.04 d
crude protein	9.85 ± 0.14 a	5.67 ± 0.22 b	7.02 ± 0.20 c	7.87 ± 0.01 d
crude fat	0.98 ± 0.02 a	0.42 ± 0.02 b	0.49 ± 0.00 c	2.30 ± 0.06 d
moisture content	4.82 ± 0.06 a	7.22 ± 0.01 b	8.12 ± 0.10 c	9.93 ± 0.21 d
total ash	3.33 ± 0.09 a	6.62 ± 0.46 b	1.79 ± 0.09 c	6.90 ± 0.11 b
fiber	36.40 ± 0.36 a	36.71 ± 0.23 a	22.60 ± 0.35 b	7.43 ± 0.43 c
energy value	372.28 ± 0.22 a	346.76 ± 1.70 b	362.83 ± 0.76 c	344.20 ± 0.71 d

[a]Results are expressed as mean ± standard deviation (n = 3). Means with different letters (a–d) within a row are significantly different ($p < 0.05$).

protocols. Total glucan and α-glucan amounts were measured by enzymatic hydrolysis; β-glucan content (g/100 g DW) was determined by difference.

RESULTS AND DISCUSSION

Comparative Chemical Composition of *L. rhinocerotis* from Different Developmental Stages. The chemical composition of fruit body (pileus and stipe), sclerotium, and mycelium of *L. rhinocerotis* was investigated. Results were compared with previous findings on other sclerotia-forming and/or wild mushrooms only.

Proximate Composition. Table 1 shows the proximate composition of *L. rhinocerotis* on a dry weight (DW) basis. Overall, the sclerotium of *L. rhinocerotis* was found to be rich in carbohydrates (82.60 g/100 g DW) but low in proteins (7.02 g/100 g DW) and fat (0.49 g/100 g DW). This is in agreement with Wong et al.,[4] who reported that the sclerotium of *L. rhinocerotis* contained higher carbohydrates (95.7 g/100 g DW) but a much lower concentration of protein (2.75 g/100 g DW) and fat (0.02 g/100 g DW). The carbohydrate level in the sclerotium of *L. rhinocerotis* was slightly lower in comparison with those of *P. tuber-regium* and *Wolfiporia cocos* (90.5–98.1 g/100 g DW) from China.[4] Furthermore, sclerotium of *P. tuber-regium* from other geographical origins (Nigeria) were reported to have low carbohydrate levels, in the range 22.2–51.8 g/100 g DW.[7,8]

The proximate composition of the fruit bodies of *L. rhinocerotis* and another polypore, i.e., a wild *Ganoderma* sp. from Nigeria, was found to be quite different despite the similarity in their woody texture.[9] Carbohydrates in the fruit body of *L. rhinocerotis* were relatively high when compared with *Ganoderma* sp. and other wild mushrooms that do not form sclerotia in their life cycle (54.1–84.5 g/100 g DW).[9−11] Carbohydrates in the pileus and stipe (fruit body) were found to be comparable to that of the sclerotium, suggesting all parts of *L. rhinocerotis* are good sources of carbohydrates. In contrast, the distribution of carbohydrates in different parts of *P. tuber-regium* was not similar, whereby Okhuoya and Ajerio[12] observed that carbohydrates in fruit bodies (30.0–38.8 g/100 g DW) were at least 2 times higher than the sclerotia (16.5–16.8 g/100 g DW), while Akindahunsi and Oyetayo[13] found that the pilei and sclerotia have higher carbohydrate levels than the stipe.

Crude protein in the sclerotium of *L. rhinocerotis* (7.02 g/100 g DW) was moderately low when compared to the sclerotia of other mushroom species. For instance, the sclerotia of cultivated or wild *P. tuber-regium* have highly variable protein contents, ranging from 13.0 to 71.2 g/100 g DW.[7,8,14,15] This might be due to the environmental conditions where the sclerotia were formed. Analyses of cultivated *P. tuber-regium*, consisting of intact fruit body and sclerotium, revealed varying protein abundance in the different parts of the mushroom. As reported by Okhuoya and Ajerio,[12] fruit bodies (9.5–10.2 g/100 g DW) were reported to have much higher protein than sclerotia (3.0–3.1 g/100 g DW). Similarly, the protein content of the pileus (9.85 g/100 g DW) of *L. rhinocerotis* was significantly higher than that of the sclerotium. Nevertheless, the higher protein distribution in the pileus compared to the stipe of *L. rhinocerotis* was consistent with previous findings on *P. tuber-regium*.[13]

All samples of *L. rhinocerotis* contained low levels of fat (0.42–2.30 g/100 g DW). Crude fat in *L. rhinocerotis* decreased in the order pileus > sclerotium > stipe, and a similar trend was also observed in *P. tuber-regium*.[13] Crude fat in the fruit body of *P. tuber-regium* was reported to be 1.7 g/100 g DW,[16] whereas in the sclerotium, the value ranged from 0.05 to 2.20 g/100 g DW.[4,8,14,15] The mycelium of *L. rhinocerotis* was found to contain significantly higher fat content than the fruit body and sclerotium. The fat content in fruit bodies varies according to the mushroom species.[9−11] In addition, Gbolagade et al.[16] reported that the fruit bodies of several wild mushrooms possessed remarkably high crude fat, up to 11.1 g/100 g DW.

Sclerotia are used in the dried form by the local and indigenous communities, and industrially, mycelia are also dried, powdered, and processed into products. To reflect the actual situation pertaining to the use of *L. rhinocerotis*, the dried form of mushroom samples was analyzed in the present study. Dried *L. rhinocerotis* possessed relatively low moisture content (<10 g/100 g DW). This agreed with previous findings on *P. tuber-regium* (6.3–10.8 g/100 g DW)[13] and *Ganoderma* sp. (10.2 g/100 g DW).[9] Moisture in the mycelium was highest, followed by the sclerotium, stipe, and pileus. This discrepancy in moisture level can be due the nature of fungal structure. Moisture contents (g/100 g DW) of the sclerotia of *P. tuber-regium, L. rhinocerotis*, and *W. cocos* were reported to be 12.9, 15.3, and 15.3, respectively.[4] The low moisture content of dried *L. rhinocerotis* and other mushroom samples gives product stability and longer storage life.

Ash represents inorganic residue left behind after incineration of samples at temperatures high enough to allow all organic matter to be burnt off. Total ash in *L. rhinocerotis* samples varied, with the mycelium and stipe having notably high amounts of ash (~7 g/100 g DW), followed by the pileus and sclerotium. Samples of *P. tuber-regium* were reported to have varying amounts of ash, and their level decreased in the following order: pileus > stipe > sclerotium.[13] Levels of ash in the sclerotia of other sclerotia-forming mushrooms were generally found to be low (<2 g/100 g DW) based on previous

findings.[4] On the other hand, the fruit bodies of wild mushrooms were noted to have high ash contents (up to 17.3 g/100 g DW).[10,11,16]

Levels of fiber in pileus and stipe of *L. rhinocerotis* in this study were considered high (~36 g/100 g DW), while the sclerotium contained significantly lower fiber content (22.6 g/100 g DW). Wong et al.[4] reported that *L. rhinocerotis* possessed remarkably high levels of insoluble dietary fiber (IDF) (87.1 g/100 g DW) and low amounts of soluble dietary fiber (SDF) (2.51 g/100 g DW). They stated that the physicochemical and functional properties of dietary fibers from *L. rhinocerotis* were comparable with some commercial dietary fiber-rich ingredients.[5] Crude fiber in other sclerotia-forming mushrooms is generally low. For instance, the crude fiber in sclerotia of *P. tuber-regium* was only in the range 3.2−16.7 g/100 g DW.[7,8,14,15] Low fiber (3.5 g/100 g DW) was also reported for *Ganoderma* sp. despite its woody, fibrous texture.[9] The mycelium of *L. rhinocerotis* was found to have significantly lower fiber content than the fruit body and sclerotium but comparable with that of the fruit bodies of several wild edible mushrooms from Southern Nigeria.[16]

Energy content of *L. rhinocerotis* (344.2−372.3 kcal/100 g DW) was found to be moderate. Our values are in agreement with the energy content values of *P. tuber-regium* sclerotia, reported in the range 322−382 kcal/100 g DW,[7,8,15] and *Ganoderma* sp., having 369.2 kcal/100 g DW.[6] The low energy content level in *L. rhinocerotis* and other mushrooms is likely attributed to low fat content.

Fatty Acid Composition. As shown in Table 2, the composition of fatty acids (% in crude fat), including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), in *L. rhinocerotis* varied with mushroom developmental stages. Overall, *L. rhinocerotis* contained higher levels of unsaturated than saturated fatty acids, with high levels of essential fatty acids. (C16:0) Palmitic acid and (C18:1) oleic acid were abundant across all samples, but both were more concentrated in the pileus and sclerotium (33.5−36.2%) compared with the stipe and mycelium (13.9−17.8%). The abundance of palmitic and oleic acids was consistent with previous findings on other wild mushrooms.[11,17]

In addition, *L. rhinocerotis* contained essential fatty acids, including short-chain PUFA, e.g., (C18:2n6c) linoleic and (C18:3n3) α-linolenic acids, as well as long-chain PUFA, e.g., (C20:5n3) eicosapentaenoic, (C22:6n3) docosahexaenoic, and (C20:4n6) arachidonic acids. The distribution of the essential fatty acids in *L. rhinocerotis* was not uniform. For instance, α-linolenic acid (0.6−7.4%) was detected in the pileus, stipe, sclerotium, and mycelium of *L. rhinocerotis*, but others were found only in certain developmental stages. Linoleic acid was found only in the stipe (33.9%) and mycelium (26.8%) of *L. rhinocerotis*. It was the predominant fatty acid in both developmental stages of this mushroom. Some high carbon fatty acids such as (C18:0) stearic, (C18:2n6c) linoleic, (C22:0) behenic, (C24:0) lignoceric, and (C24:1) nervonic acids were abundant in the mycelium of *L. rhinocerotis*. However, the mycelia were devoid of (C4:0) butyric and (C6:0) caproic acid, which were present only in the pileus and sclerotium. The sclerotium was lacking in (C11:0) undecanoic and linoleic acids. The fatty acid profile of the pileus showed some similarities to the mycelium. Previously, fruit bodies and mycelia of *Tuber indicum* and *Tuber aestivum* were reported to have nearly identical fatty acid profiles.[17] It was also noted that the fruit bodies, sclerotium, and mycelium of *L. rhinocerotis* were free from trans-fatty acids.

Table 2. Fatty Acid Profiles of the Fruit Body, Sclerotium, and Mycelium of *L. rhinocerotis*[a]

		fruit body			
fatty acid content (% in crude fat)		pileus	stipe	sclerotium	mycelium
C4:0	butyric	0.05	0.00	0.05	0.00
C6:0	caproic	0.07	0.00	0.06	0.00
C8:0	caprylic	0.36	0.11	0.35	0.10
C10:0	capric	0.37	0.11	0.35	0.11
C11:0	undecanoic	0.02	0.00	0.00	0.04
C12:0	lauric	3.71	1.05	3.61	1.14
C13:0	tridecanoic	0.02	0.00	0.02	0.05
C14:0	myristic	2.75	0.96	2.57	0.68
C14:1	myristoleic	0.10	1.11	0.15	0.12
C15:0	pentadecanoic	0.20	1.12	0.12	0.24
C15:1	*cis*-10-pentadecanoic	0.05	0.22	0.02	0.06
C16:0	palmitic	33.78	17.49	36.21	13.89
C16:1	palmitoleic	0.50	0.59	0.37	0.27
C17:0	heptadecanoic	0.18	0.88	0.15	0.18
C17:1	*cis*-10-heptadecanoic	0.14	0.88	0.08	0.18
C18:0	stearic	4.93	4.51	5.34	5.09
C18:1n9	elaidic (trans)	0.00	0.00	0.00	0.00
C18:1n9c	oleic	33.47	17.74	34.44	17.02
C18:2n6	linolelaidic (trans)	0.00	0.00	0.00	0.00
C18:2n6c	linoleic (cis)	0.00	33.92	0.00	26.79
C18:3n6	γ-linolenic	12.02	0.00	7.85	0.15
C18:3n3	α-linolenic	0.68	2.57	0.59	7.34
C20:0	arachidic	0.67	0.41	0.71	0.32
C20:1n9	*cis*-11-eicosenoic	0.40	0.26	0.31	0.22
C20:2	*cis*-11,14-eicosadienoic	0.05	0.52	0.02	0.21
C20:3n6	*cis*-8,11,14-eicosatrienoic	0.05	0.00	0.02	0.18
C20:3n3	*cis*-11,14,17-eicosatrienoic	0.14	0.00	0.13	0.07
C21:0 + C20:4n6	henicosanoic + arachidonic	0.19	0.63	0.06	0.41
C20:5n3	*cis*-5,8,11,14,17-eicosapentaenoic	0.77	2.03	0.13	3.06
C22:0	behenic	0.91	2.22	0.88	6.04
C22:1n9	erucic	0.28	0.49	0.37	0.61
C22:2	cis-13,16-docosadienoic	0.25	0.00	0.29	0.84
C23:0	tricosanoic	0.39	1.48	0.44	0.66
C22:6n3	*cis*-4,7,10,13,16,19-docosahexaenoic	0.11	0.00	0.07	1.35
C24:0	lignoceric	1.67	4.38	1.22	5.74
C24:1	nervonic	0.72	4.32	3.07	6.83

[a]Results are expressed as percentage of fatty acid in crude fat.

Mineral Content. Samples of *L. rhinocerotis* contained different levels of macro- and microelements, as shown in Table 3. Potassium was found to be the most abundant mineral in all mushroom samples, with mycelium (~1.4 g/100 g DW) recording a significantly higher concentration than the fruit body and sclerotium. The mycelium of *L. rhinocerotis* also contained the highest amounts of magnesium, phosphorus, and sodium. The sclerotium of *L. rhinocerotis* contained higher potassium content than the fruit bodies, but other minerals

Table 3. Mineral and Vitamin Contents (mg/100 g DW) in the Fruit Body, Sclerotium, and Mycelium of *L. rhinocerotis*[a]

parameter	fruit body		sclerotium	mycelium
	pileus	stipe		
Macroelements				
potassium	178.10 ± 6.35 ab	118.73 ± 7.44 a	225.00 ± 6.92 b	1412.83 ± 85.41 c
phosphorus	163.69 ± 8.39 a	67.17 ± 6.46 b	114.90 ± 2.13 ab	1002.23 ± 63.60 c
magnesium	99.29 ± 2.74 a	42.00 ± 3.75 b	64.77 ± 6.13 c	171.75 ± 8.20 d
calcium	79.87 ± 0.93 a	195.15 ± 6.10 b	76.73 ± 2.38 a	55.64 ± 1.19 c
sodium	7.04 ± 0.33 a	4.40 ± 0.26 a	3.96 ± 0.47 a	104.25 ± 4.97 b
Microelements				
iron	31.91 ± 1.23 a	100.77 ± 2.55 b	12.93 ± 0.21 c	3.34 ± 0.18 d
zinc	4.73 ± 0.13 a	1.82 ± 0.08 b	1.19 ± 0.04 c	7.36 ± 0.44 d
manganese	0.48 ± 0.03 a	0.45 ± 0.00 a	0.24 ± 0.01 b	0.28 ± 0.01 c
copper	1.85 ± 0.04 a	0.83 ± 0.01 b	0.59 ± 0.06 b	4.73 ± 0.42 c
#selenium (μg/100 g)	11.00 ± 0.00 a	6.00 ± 0.00 b	9.00 ± 0.00 b	13.00 ± 0.00 a
Vitamins				
thiamine (vitamin B_1)	N. D.	N. D.	N. D.	N. D.
riboflavin (vitamin B_2)	0.61 ± 0.00 a	0.19 ± 0.00 b	0.06 ± 0.00 c	1.72 ± 0.01 d
niacin (vitamin B_3)	23.54 ± 0.36 a	N. D.	N. D.	141.53 ± 1.92 b
pantothenic acid (vitamin B_5)	N. D.	N. D.	N. D.	N. D.
pyridoxine (vitamin B_6)	N. D.	N. D.	N. D.	N. D.
folic acid (vitamin B_9)	N. D.	N. D.	N. D.	N. D.

[a]Results are expressed as mean ± standard deviation (n = 3). Means with different letters (a–d) within a row are significantly different ($p < 0.05$). #Values for selenium were expressed as μg selenium/100 g DW mushroom samples. N.D. (not detected). Detection limit for the vitamins are as follows: thiamine (<0.05 mg/100 g DW), niacin (<0.10 mg/100 g DW), pantothenic acid (<0.10 mg/100 g DW), pyridoxine (<0.06 mg/100 g DW), and folic acid (0.06 mg/100 g DW). The level of niacin in the pileus and mycelium is significantly different ($p < 0.05$) based on t-test.

were more concentrated in either the pileus or stipe. Similarly, only sodium was more concentrated in the sclerotium of *P. tuber-regium*, while other minerals were more abundant in the fruit body.[13] Among the microelements, iron was the most abundant mineral in *L. rhinocerotis* samples, ranging from 3.3 to 100.8 mg/100 g DW. Mycelium have the highest concentrations of zinc and copper, whereas iron and manganese were concentrated in the fruit body. All mushroom samples contained extremely low (~0.01 mg/100 g) amounts of selenium.

All minerals determined were present in *L. rhinocerotis* from all developmental stages. In *P. tuber-regium*, the distribution of minerals in the fruit body and sclerotium was not uniform. In their study, Akindahunsi and Oyetayo[13] did not detect magnesium, copper, and zinc in the sclerotium of *P. tuber-regium*. They, however, reported extremely low amounts (0.3–5.0 mg/100 g) of the same minerals in the pileus and stipe.

Vitamin Content. While mushrooms have been considered to be a good source for most of the vitamin B complexes, *L. rhinocerotis* samples contain very low levels of these, as depicted in Table 3. Most were absent (below detection limit) except for riboflavin (vitamin B_2) and niacin (vitamin B_3). Riboflavin (0.1–1.7 g/100 g DW) was found in all samples. Only the mycelium and pileus of *L. rhinocerotis* contained niacin, and its concentration in the mycelium (141.5 g/100 g DW) was significantly higher than that of the pileus (23.5 g/100 g DW).

β-Glucans. The level of β-glucans from different developmental stages of *L. rhinocerotis* was comparable, ranging from 9.3 to 13.2 g/100 g DW, as shown in Figure 2. There was no significant difference ($p < 0.05$) in the levels of β-glucans in the pileus, stipe, sclerotium, and mycelium of *L. rhinocerotis*. Our results for the sclerotium of *L. rhinocerotis* were in agreement with that of Wong and Cheung,[5] who reported that sclerotial

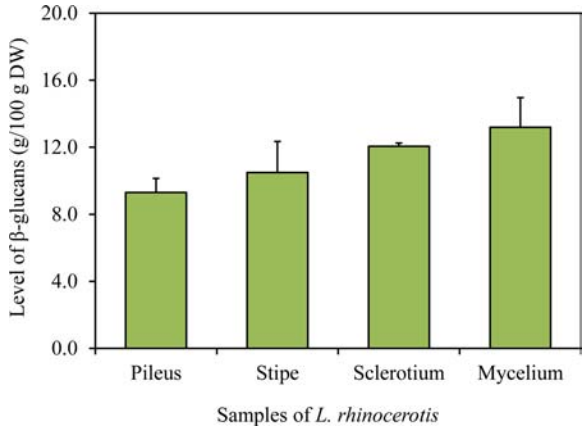

Figure 2. Level of β-glucans in *L. rhinocerotis*. Results are expressed as mean ± standard deviation (n = 3).

dietary fiber of *P. tuber-regium*, *L. rhinocerotis*, and *W. cocos* was rich in β-glucans with a very low level of resistant glycogen. Levels of β-glucans in *L. rhinocerotis*, on the other hand, were lower than some edible mushrooms such as shitake, maitake, and dried black fungus, which contained β-glucan in the range 38.6–48.3 g/100 g DW (Megazyme International Ireland).

Distribution of Chemical Components of *L. rhinocerotis* in Relation to Developmental Stages, Physiological Roles, and Cultivation Techniques. Plausible reasons for the varying distribution of some chemical components in different parts of the fruit body, sclerotium, and mycelium of *L. rhinocerotis* were deduced based on our findings and literature available. Differences in the chemical composition of *L. rhinocerotis* were likely attributed to the distinct developmental/physiological stages aforementioned. Selective accumulation

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of certain chemical components might also be linked to specific functional and/or physiological roles in fungal developmental and nutritional aspects.

Mushroom sclerotia contained high levels of food reserves to support the growth of new fruit bodies and spore release for survival. As such, this could explain the high level of carbohydrates (starch, glycogen, reducing and nonreducing sugars) in the sclerotia of *L. rhinocerotis* and other mushrooms previously reported.[4] According to Willets and Bullock,[18] the main cytoplasmic reserves in fungal sclerotia include glycogen, protein, and lipids. The inverse relationship between the level of carbohydrates and lipids in the fungi has been noted.[10,11] Higher lipid content in mycelia than that of the fruit bodies might be partly due to the mycelia being harvested during the vegetative stage, which was speculated as the energy storage for fungal maturation.[17]

Previous investigations have shown that fungal proteins of mycelia origin were distinct from sclerotial proteins.[19] Proteomic analysis of *P. tuber-regium* demonstrated the roles of various cell wall proteins at various developmental stages,[20] reflecting the regulation of protein expression to accommodate physiological changes at each stage. In our study, the amino acid composition of *L. rhinocerotis* varied according to developmental stages (Supporting Information), suggesting a different array of proteins might be present.

The level of nutrients in the pileus and stipe of *L. rhinocerotis* showed some variations. According to Ola and Oboh,[21] higher nutrients in the pileus than the stipe could be associated with the role of the pileus in spore production, leading to an increase in nutrient requirement and subsequent accumulation of nutrients in the pilei. Another hypothesis put forward by Fasidi and Kadiri[22] was that a strong metabolic force from the pileus draws materials continuously from the substrate through the stipe during development of the fruit body. According to Fasidi and Kadiri,[22] mushroom stipes are rich in fibers, as they provide structural support for the pilei. However, no significant difference in the amount of fiber in the pileus and stipe of *L. rhinocerotis* was observed.

Accumulation of minerals in mushrooms is due to uptake from the substrates. This process can be affected by factors such as fungal strain, physiological status and pH of the substrate, and bioavailability of the minerals.[23] Fruit bodies and mycelia may have different rates of mineral uptake, as demonstrated by Tong et al.[24] Therefore, it is feasible to suggest that differences in the level of minerals in *L. rhinocerotis* were a consequence of both internal and external factors.

Chemical composition of *L. rhinocerotis* mycelium produced from submerged fermentation is largely dependent on the cultivation media. The abundance of potassium and phosphorus was likely a consequence of the high level of salts KH_2PO_4 and K_2HPO_4. Potassium and phosphate ions were actively absorbed into fungal cells. However, translocation of similar minerals into the fruit bodies and sclerotia was dependent on the soil where the substrate bags were buried. Culture conditions also affect chemical profiles. Previously, some differences were noted in protein profiles of *L. rhinocerotis* cultivated using two methods of liquid fermentation.[3] The effect of environmental and cultivation conditions on the chemical composition of *L. rhinocerotis* should be investigated further in order to maximize the production of chemical components of interest.

Potential Utilization of *L. rhinocerotis* As Nutraceuticals Based on Nutritional Content and Bioactive Constituents. Information on the chemical composition of *L. rhinocerotis* is vital for further development of the mushroom as a source of nutraceuticals. With reference to the chemical composition, *L. rhinocerotis* is aptly utilized as a dietary supplement, fitting the nutritional requirements of various individuals. A high level of carbohydrates, a moderate level of proteins, and very low level of lipids are suitable for those under diet restrictions especially diabetes patients. Besides, *L. rhinocerotis* is also rich in dietary fiber, which is beneficial to intestinal function by increasing fecal bulk and enhancing peristalsis.[7] A high ratio of potassium to sodium renders *L. rhinocerotis* suitable for hypertension and heart disease patients.[11] In view of the low lipid content of *L. rhinocerotis*, it can be incorporated into components of a low-fat diet for those who are on a weight management program.[10] Due to its protein content, *L. rhinocerotis* is a suitable source of protein supplementation. Mushroom proteins are generally deemed of superior quality due to the presence of all essential amino acids.[14] Preliminary results indicated that *L. rhinocerotis* contains varying levels of all essential amino acids except tryptophan. Furthermore, *L. rhinocerotis* contains appreciable amounts of essential fatty acids. The importance of these fatty acids in human metabolism has been described elsewhere.[11,17] Although being considered as nonessential, some fatty acids have important physiological roles. For example, oleic acid decreases cholesterol and reduces the incidence of cardiovascular diseases.[11] Samples of *L. rhinocerotis*, especially mycelium, were rich in riboflavin and niacin, which are important in human metabolism.

From the medicinal point of view, earlier findings have revealed that polysaccharides might be responsible for the therapeutic effect of *L. rhinocerotis*, e.g., anticancer potential via immunomodulatory effect.[25] From our results, the fruit body and mycelium of *L. rhinocerotis* also contained high levels of carbohydrates; hence, it will be interesting to investigate if similar polysaccharides are produced when the mushroom is cultured under submerged fermentation and whether these polysaccharides possessed similar bioactivities. Among the fungal polysaccharides, β-glucans were regarded as important bioactive constituents responsible for various health-promoting effects including cholesterol-lowering, antitumor, and immunomodulatory. It will be worthwhile to isolate and characterize the β-glucans in the mycelium of *L. rhinocerotis*.

***Lignosus rhinocerotis* As a Source of Functional Ingredients with Consideration of Mushroom Parts and Cultivation Techniques.** Due to selective accumulation of nutrients in different fungal tissues, it would be practical to choose the parts that contain the highest levels of desired nutrients to harness the potential of *L. rhinocerotis* to the fullest. The fruit body, sclerotium, and mycelium of *L. rhinocerotis* were obtained from different cultivation techniques, i.e., solid-substrate and submerged fermentation, which differ in terms of cost, time, and space required. These factors should be taken into consideration as well.

The fruit body and sclerotium of *P. tuber-regium* were reported to be edible.[14] The fungal tissue of *P. tuber-regium* was soft as compared with the woody nature of *L. rhinocerotis*. To the best of our knowledge, this mushroom is used as a folk medicine and not eaten as a source of food. Similar to "lingzhi" (*Ganoderma* spp.), *L. rhinocerotis* is also regarded as "nonedible" due to the texture of the mushroom itself. Nevertheless, both mushrooms, appreciated for their medicinal properties, represent potential sources of bioactive constituents. Thus, *L.*

rhinocerotis might not be taken directly as food, but it can be developed as a new source of functional ingredients such as dietary fiber with various physiological benefits.

The chemical composition of the pilei and stipes of several mushrooms varied as discussed earlier. The pilei are usually superior in terms of nutrients, but the stipe contained a higher fraction of insoluble crude fiber. The stipe of a mushroom is often underutilized in food processing and discarded.[26] On the basis of our findings and results from previous investigations,[4,5] the stipe and sclerotium of *L. rhinocerotis* should be further exploited since these are rich in dietary fibers. Depending on the physicochemical properties, fiber-rich *L. rhinocerotis* can be used as food additives in the formulation of food products that require foaming, emulsification, gel formation, and retention of flavor and palatability.[9] Sclerotial flour of *P. tuber-regium* has been studied as a food additive due to its binding properties.[7]

Mycelium of *L. rhinocerotis* contained some distinctively different chemical components compared with the fruit body and sclerotium. This means that the mycelium of *L. rhinocerotis* represents a viable source of nutraceuticals, and submerged fermentation might be an alternative for the production of fungal biomass. During submerged fermentation, various factors such as temperature, light, pH, aeration, and nutrients in the media could affect fungal growth and their chemical composition.[3] With good understanding of biosynthetic pathways, these could be manipulated and further optimized in order for the fungal mycelia to accumulate the desired chemical components. Some have demonstrated that mycelia possessed a higher rate of mineral uptake as compared with the fruit bodies.[24] For *L. rhinocerotis*, the submerged culture has a shorter growing period (15 days)[3] than the fruit body (approximately 12−15 months).[2] Cultivation in the form of mycelia was considered to be relatively simpler, more economical, fast, less problematic, and easier to manipulate.

Our findings on various samples of *L. rhinocerotis* debunked the belief that only the sclerotium of *L. rhinocerotis* is important from the nutritional and medicinal points of view. Indeed, the sclerotium were clearly not superior to other parts of the fruit body, and mycelium could emerge as an alternative choice taking into account some of the advantages conferred by submerged fermentation. Only the sclerotia are being sought after, while the fruit bodies are generally neglected. These underutilized parts must be further investigated to optimize the usage and potential of *L. rhinocerotis*. Attention must also be given to the production of mycelium through submerged fermentation for reasons explained above.

It is to be emphasized again that the chemical compositions of the fruit body, sclerotium, and mycelium of *L. rhinocerotis* were distinctively different. Differential accumulation of certain desired chemical components might pave the way for future development of nutraceuticals from *L. rhinocerotis*. Our findings are by no means exhaustive, but these provide insights into nutritional status and potential nutraceutical applications of *L. rhinocerotis*. Additional information, e.g., mycochemical profiles and bioactivity evaluation, would further substantiate the medicinal values of *L. rhinocerotis* from different developmental stages.

■ ASSOCIATED CONTENT

Supporting Information

Preliminary analysis of essential amino acid composition in fruit body, sclerotium, and mycelium of *L. rhinocerotis*. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: noorlidah@um.edu.my. Tel: +603-7967 4371. Fax: +603-7967 4178.

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■ REFERENCES

(1) Lee, S. S.; Chang, Y. S. Ethnomycology. In *Malaysian Fungal Diversity*; Jones, E. B. G., Hyde, K. D., Sabaratnam, V., Eds.; Mushroom Research Centre, University of Malaya and Natural Resources and Environment, Malaysia: Malaysia, 2007; pp 307−317.

(2) Abdullah, N.; Dzul Haimi, M. Z.; Lau, B. F.; Annuar, M. S. Domestication of a wild medicinal sclerotial mushroom, *Lignosus rhinocerotis* (Cooke) Ryvarden. *Ind. Crops Prod.* **2013**, *47*, 256−261.

(3) Lau, B. F.; Aminudin, N.; Abdullah, N. Comparative SELDI-TOF-MS profiling of low-molecular-mass proteins from *Lignosus rhinocerus* (Cooke) Ryvarden grown under stirred and static conditions of liquid fermentation. *J. Microbiol. Methods* **2011**, *87*, 56−63.

(4) Wong, K. H.; Cheung, P. C. K.; Wu, J. Z. Biochemical and microstructural characteristics of insoluble and soluble dietary fiber prepared from mushroom sclerotia of *Pleurotus tuber-regium*, *Polyporus rhinocerotis*, and *Wolfiporia cocos*. *J. Agric. Food Chem.* **2003**, *51*, 7197−7202.

(5) Wong, K.-H.; Cheung, P. C. K. Dietary fibers from mushroom sclerotia: 1. Preparation and physicochemical and functional properties. *J. Agric. Food Chem.* **2003**, *53*, 9395−9400.

(6) Staffas, A.; Nyman, A. Determination of cholecalciferol (vitamin D_3) in selected foods by liquid chromatography: NMKL collaborative study. *J. AOAC Int.* **2003**, *86*, 400−406.

(7) Alobo, A. P. Proximate composition and functional properties of *Pleurotus tuberregium* sclerotia flour and protein concentrate. *Plant Foods Hum. Nutr.* **2003**, *58*, 1−9.

(8) Ikewuchi, C. C. I.; Ikewuchi, J. G. Chemical profile of *Pleurotus tuber-regium* (Fr) Sing's sclerotia. *Pac. J. Sci. Technol.* **2008**, *10*, 295−299.

(9) Aremu, M. O.; Basu, S. K.; Gyar, S. D.; Goyal, A.; Bhowmik, P. K.; Datta, B. Proximate composition and functional properties of mushroom flours from *Ganoderma* spp., *Omphalotus olearius* (DC.) Sing. and *Hebeloma mesophaeum* (Pers.) Quel. used in Nasarawa State, Nigeria. *Malays. J. Nutr.* **2009**, *15*, 233−241.

(10) Chong, K. S.; Chye, F. Y.; Lee, J. S.; Atong, M. Nutritional properties of some edible wild mushrooms in Sabah. *J. Appl. Sci.* **2007**, *7*, 2216−2221.

(11) Liu, Y.-T.; Sun, J.; Luo, Z.-Y.; Rao, S.-Q.; Su, Y.-J.; Xu, R.-R.; Yang, Y. J. Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. *Food Chem. Toxicol.* **2012**, *50*, 1238−1214.

(12) Okhuoya, J. A.; Ajerio, C. Analysis of sclerotia and sporophore of *Pleurotus tuber-regium* Fr. An edible mushroom in Nigeria. *Korean J. Mycol.* **1988**, *16*, 204−206.

(13) Akindahunsi, A. A.; Oyetayo, F. L. Nutrient and antinutrient distribution of edible mushroom, *Pleurotus tuber-regium* (Fries) Singer. *LWT—Food Sci. Technol.* **2006**, *39*, 548—553.
(14) Fasidi, I. O.; Ekuere, U. U. Studies on *Pleurotus tuber-regium* (Fries) Singer: cultivation, proximate composition and mineral contents of sclerotia. *Food Chem.* **1993**, *48*, 255—258.
(15) Nwokolo, E. Composition of nutrients in the sclerotium of the mushroom *Pleurotus tuber regium*. *Plant Foods Hum. Nutr.* **1987**, *37*, 133—139.
(16) Gbolagade, J.; Ajayi, A.; Oku, I.; Wankasi, D. Nutritive value of common wild edible mushrooms from Southern Nigeria. *Global J. Biotechnol. Biochem.* **2006**, *1*, 16—21.
(17) Tang, Y.; Li, Y.-Y.; Li, H.-M.; Wan, D.-J.; Tang, Y.-J. Comparison of lipid content and fatty acid composition between *Tuber* fermentation mycelia and natural fruiting bodies. *J. Agric. Food Chem.* **2011**, *59*, 4736—4742.
(18) Willets, H. J.; Bullock, S. Developmental biology of sclerotia. *Mycol. Res.* **1992**, *96*, 801—816.
(19) Petersen, G. R.; Russo, G. M.; van Etten, J. L. Identification of major proteins in sclerotia of *Sclerotinia minor* and *S. trifoliorum*. *Exp. Mycol.* **1982**, *6*, 268—273.
(20) Chen, L.; Zhang, B.-B.; Cheung, P. C. K. Comparative proteomic analysis of mushroom cell wall proteins among the different developmental stages of *Pleurotus tuber-regium*. *J. Agric. Food Chem.* **2012**, *60*, 6173—6182.
(21) Ola, F. L.; Oboh, G. Nutrient distribution and zinc bioavailability: estimation in some tropical edible mushrooms. *Nahrung/Food* **2001**, *45*, 67—68.
(22) Fasidi, I. O.; Kadiri, M. Effect of sporophore maturity on chemical composition of *Volvariella esculenta* (Mas) Singer, a Nigerian mushroom. *Die Nahrung.* **1993**, *37*, 269—273.
(23) Kalač, P.; Svoboda, L. A review of trace element concentrations in edible mushrooms. *Food Chem.* **2000**, *69*, 273—281.
(24) Tong, C. C.; Khoong, S. L.; Lee, C. K. Germanium uptake by the fruiting bodies and mycelium of the fungus *Ganoderma lucidum*. *ASEAN Food J.* **1994**, *9*, 69—72.
(25) Wong, K.-H.; Lai, C. K. M.; Cheung, P. C. K. Immunomodulatory activities of mushroom sclerotial polysaccharides. *Food Hydrocolloids* **2011**, *25*, 150—158.
(26) Zhang, Z.; Song, H.; Peng, Z.; Luo, Q.; Ming, J.; Zhao, G. Characterization of stipe and cap powders of mushroom (*Lentinus edodes*) prepared by different grinding methods. *J. Food Eng.* **2012**, *109*, 406—413.