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Determination of Mineral Components in the Cultivation Substrates of Edible Mushrooms and Their Uptake into Fruiting Bodies

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The mineral contents of the cultivation substrates, fruiting bodies of the mushrooms, and the postharvest cultivation substrates were determined in cultivated edible mushrooms *Pleurotus eryngii*, *Flammulina velutipes*, and *Hypsizigus marmoreus*. The major mineral elements both in the cultivation substrates and in the fruiting bodies were K, Mg, Ca, and Na. Potassium was particularly abundant ranging 10~13 g/kg in the cultivation substrates and 26~30 g/kg in the fruiting bodies. On the contrary, the calcium content in the fruiting bodies was very low despite high concentrations in the cultivation substrates, indicating Ca in the cultivation substrates is in a less bio-available form or the mushrooms do not have efficient Ca uptake channels. Among the minor mineral elements determined in this experiment, Cu, Zn, and Ni showed high percentage of transfer from the cultivation substrates to the fruiting bodies. It is noteworthy that the mineral contents in the postharvest cultivation substrates were not changed significantly which implies that the spent cultivation substrates are nutritionally intact in terms of mineral contents and thus can be recycled as mineral sources and animal feeds.

KEYWORDS : Edible mushroom, *Flammulina*, *Hypsizigus*, Mineral, *Pleurotus*, Substrate

Mushrooms belong to fungal groups that form characteristic fruiting bodies in which spores reside. They not only play an important role in the recycling of plant materials in an ecosystem but also are appreciated as good sources of food and medicine. Edible mushrooms, *Agaricus bisporus* and *Pleurotus ostreatus*, are popular mushrooms with high commercial values and are thus cultivated world wide. *Pleurotus eryngii*, *Flammulina velutipes*, and *Hypsizigus marmoreus* are edible mushrooms particularly popular in East Asia, cultivation of which are facilitated using specially formulated substrates in semi-automated cultivation facilities. The former two are generally cultivated on a culture bed consisting of dairy manure-wheat straw or dairy manure-rice straw composts. The latter three are cultivated in wide-mouth polypropylene bottles which contain a substrate mixture constituted of various agricultural wastes including rice bran, corncob, soybean hull, and sawdust. In either case, proper composition of the cultivation substrate with good preparation practice is crucial for the reliable production of mushrooms. For example, basal substrate supplemented with Mn and ground soybean resulted in significant enhancement in the production yield of *P. eryngii* (Rodriguez Estrada and Royse, 2006). Addition of limiting mineral components promoted mycelia growth rate of *P. ostreatus* up to 25% (Curvetto *et al.*, 2002). Because mycelia propagation within the cultivation substrate takes at least a month to cultivate these mushrooms, an enhancement in the mycelia growth rate by 25% means a huge reduction in production cost.

In this report, we determined the mineral contents of the cultivation substrates for the mushrooms *P. eryngii*, *F. velutipes*, and *H. marmoreus*. The percentage of mineral transfer from the cultivation substrate to the mushroom fruiting body was assessed by comparing the mineral contents in the fruiting bodies with those in the input substrates. We also determined the mineral contents in the postharvest cultivation substrates which are often recycled for an additional round of cultivation and are utilized as animal feed sources (Kim *et al.*, 2007b; Kwak *et al.*, 2008).

Materials and Methods

Substrate composition and mushroom cultivation conditions. The substrate for *H. marmoreus* consisted of pine sawdust (23%), concob (32%), rice bran (32%), and soybean hull (22%). The cultivation of *H. marmoreus* was carried out at 15°C in an incubating room with 3000~4000 ppm CO_2 and 95% relative humidity (RH). The substrate for *P. eryngii* was rather complex. It contained pine sawdust (23%), concob (29%), rice bran (18%), beet pulp (4%), wheat bran (14%), cottonseed hull (4%), and shell powder (4%), and dehydrated beverage by-product from soybean (14%). The cultivation conditions for *P. eryngii* were similar to *H. marmoreus* with slight differences: 500~2000 ppm CO_2 and 87% RH. *F. velutipes* was cultured at 5°C with 3000~5000 ppm CO_2 and 90% RH. Its substrate consisted of concob (31%), rice bran (40%), beet

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pulp (16%), wheat bran (5%), cottonseed hull (5%), and shell powder (4%). The total growth period for *H. marmoreus*, *P. eryngii*, and *F. velutipes* was 23 days, 18 days, and 30 days, respectively. Each individual component in the cultivation substrate was measured as weight percent (wt%).

Sample preparation. The fruiting bodies, cultivation substrates, and postharvest cultivation substrates of *H. marmoreus*, *F. velutipes*, and *P. eryngii* were collected from mushroom farms in southern Korea. The samples (50.0 g) were dried at 100°C for 48 hrs and their dry weights were determined. The dried sample was ground using a mortar and pestle. For complete digestion, the ground sample was digested by a solution containing a 10 : 1 mixture of 50% HNO_3 and 30% H_2O_2 solutions (digestion solution) as previously described (Gergely *et al*., 2006). Briefly, the sample solution containing 0.5 g of the ground powder in 100 *ml* of the digestion solution was heated for 24 hrs. When the solution became clear with slightly yellowish color, the digestion was stopped and the volume of the sample solution was adjusted to 40 *ml* with deionized water.

Quantification of mineral elements in the cultivation substrates and fruiting bodies. In order to investigate the distribution of minerals and trace elements in mushroom production, we determined the concentration of mineral components in the fruiting bodies of mushrooms and their cultivation substrates. The digested sample solutions were subjected to inductively coupled plasma spectrometry (ICP spectrometer Optima 5300DV, Perkin Elmer, CT, USA) or ICP mass spectrometry (ICP-MS, Elan DRC II, Perkin Elmer). Mineral elements including Na, K, Ca, Mg, Al, Mn, and Fe were quantified by ICP. Other trace elements including Cu, Zn, Pb, Ni, and Se were quantified by ICP-MS. The results are summarized

Table 1. The determination of dry weight of mushroom fruiting bodies and cultivation substrates

	Fruiting body (50 g)		Substrate (50 g)		Post-harvest substrate (50g)	
	Dry weight[a] (g)	Water content (%)	Dry weight (g)	Water content (%)	Dry weight (g)	Water content (%)
H. marmoreus	6.1 ± 0.1	87.8	18.1 ± 0.5	63.8	18.7 ± 0.8	62.6
F. velutipes	8.3 ± 0.2	83.4	16.6 ± 0.7	66.8	20.1 ± 0.5	59.8
P. eryngii	6.8 ± 0.2	86.4	17.1 ± 0.4	65.8	16.1 ± 0.5	67.8

[a]The measurement was triplicated. Data are expressed as mean ± SEM (standard error of the mean).

Table 2. The production yield of mushroom fruiting bodies

	Substrate input (g)		Harvested fruiting body (g)		Yield (B/A, %)
	Wet weight[a]	Dry weight (A)	Wet weight	Dry weight (B)	
H. marmoreus	556.0 ± 1.2	201.3	180.2 ± 2.5	21.9	10.9
F. velutipes	748.3 ± 4.1	248.3	289.6 ± 2.7	48.1	19.4
P. eryngii	840.4 ± 3.4	287.3	199.8 ± 2.8	27.2	9.6

[a]The wet weight of 5 samples per each mushroom was measured separately and expressed as mean ± SEM.

Table 3. The mineral concentrations (mg/kg) of dried mushroom fruiting bodies and cultivation substrates[a]

	H. marmoreus		*F. velutipes*		*P. eryngii*	
	Substrate	Fruiting body	Substrate	Fruiting body	Substrate	Fruiting body
Na	220.3	61.3	563.8	187.8	702.1	253.6
K	11417.1	30017.1	13465.1	28009.1	10089.1	26273.1
Ca	5071.4	159.8	19647.4	324.3	15927.4	162.5
Mg	3641.0	962.6	5417.8	1108.2	3072.2	1233.0
Al	336.3	0	347.2	8.2	218.1	41.5
Mn	106.1	12.5	154.1	7.0	99.4	7.1
Fe	507.3	62.7	410.5	108.8	292.0	39.0
Cu	5.6	2.2	7.2	2.7	4.5	6.6
Zn	35.2	36.9	37.1	27.2	28.6	52.2
Ni	0.760	1.653	0.910	0.104	1.325	0.065
Se	0.657	0.252	0.049	0	0.090	0.078
Pb	0.150	0	1.148	0	0.191	0.014

[a]The measurement was carried out 5 times. Data in the table are mean values of the measured data. Standard error of the mean (SEM) of each data was less than 1% of the mean value. SEM values are omitted for the clarity in this table.

Table 4. The transfer rate of minerals from the cultivation substrate to the fruiting bodies

	H. marmoreus	*F. velutipes*	*P. eryngii*
Na	3.4	8.1	3.6
K	31.7	50.5	25.7
Ca	0.4	0.4	0.1
Mg	3.2	5.0	4.0
Al	0	0.6	1.9
Mn	1.4	1.1	0.7
Fe	1.5	6.4	1.3
Cu	4.7	9.2	14.6
Zn	12.6	17.8	18.0
Ni	26.2	2.8	0.5
Se	4.6	0	8.6
Pb	0	0	0.7

in Tables 3~5. The concentrations of mineral components in the dried samples in mg/kg (part per million, ppm) unit are shown in Table 3. The transfer rate of minerals from the cultivation substrate to the fruiting body is shown in Table 4. This rate was calculated based upon the concentration of a mineral (mg/kg) per cultivated fruiting bodies (kg) divided by the concentration of the mineral (mg/kg) per cultivation substrate input (kg). For example, the rate of transferred Na for *H. marmoreus* was (61.3 mg/kg × 0.024 kg)/(220.3 mg/kg × 0.199 kg) × 100 = 3.4%. The amounts for the cultivation substrate inputs and the cultivated fruiting bodies are shown in Table 2. The concentrations of mineral components in wet samples are reconstituted using the water contents in Table 1 and the mineral contents in dried samples shown in Table 3.

Results and Discussion

Determination of mushroom production yields in dry weight. The edible mushrooms *H. marmoreus*, *F. velutipes*, and *P. eryngii* are cultivated through formulated substrates which are contained in wide-mouth polypropylene bottles with a volume capacity × diameter of 850 *ml* × 58 mm for *H. marmoreus*, 1030 *ml* × 75 mm for *F. velutipes*, and 1280 *ml* × 80 mm for *P. eryngii* in commercial farms. In order to investigate the mushroom yield per cultivation substrate input, we measured the dry weights of the fruiting bodies and the cultivation substrates. As shown in Table 1, the water concentration of the fruiting bodies was 87.8, 83.4, and 86.4% for *H. marmoreus*, *F. velutipes*, and *P. eryngii*, respectively, while the water concentration of the cultivation substrates was 63.8, 66.8, and 65.8%, respectively. The mushroom farms collected 180 g (22.0 g dry weight), 290 g (48.1 g dry weight), and 200 g (27.2 g dry weight) of the fruiting bodies for *H. marmoreus, F. velutipes,* and *P. eryngii,* respectively, from 556 g (201.3 g dry weight), 748 g (248.2 g dry weight), and 840 g (287.3 g dry weight) of the cultivation substrate for each corresponding mushroom. From these figures, the production yields in dry weight are 10.9% for *H. marmoreus*, 19.4% for *F. velutipes*, and 9.6 % for *P. eryngii* (Table 2). Therefore, it is likely that the cultivation substrate for *F. velutipes* contains a better formulation compared with that for *H. marmoreus* and *P. eryngii* in terms of efficient utilization of resources.

Major mineral elements in the cultivation substrates and the fruiting bodies. K, Ca, Mg, and Na were the major mineral elements found in the cultivation substrates and in the fruiting bodies regardless of mushroom species (Table 3). Potassium, which is commonly found in other cultivated or wild mushrooms (Mattila *et al.*, 2001; La Guardia *et al.*, 2005), was particularly abundant in the cultivation substrates and fruiting bodies for all 3 mushrooms. The concentration of K in the mushroom fruiting bodies was as much as 26~30 g/kg dry weight. K in the cultivation substrate transferred very efficiently to the fruiting body. The transfer rate of K was 31.7, 50.5, and

Table 5. The mineral concentration (mg/kg) of the postharvest cultivation substrates

	H. marmoreus[a]	*F. velutipes*[a]	*P. eryngii*[a]	Corn[b]	Barley[b]	Wheat[b]
Na	546.4	994.1	769.3	200	200	800
K	11097.1	11249.1	10449.1	5000	6200	6600
Ca	9127.4	25111.4	20119.4	200	700	500
Mg	5068.2	8030.6	3362.6	1000	1600	1400
Al	1750.8	377.5	292.9			
Mn	164.6	187.3	109.4	23	38	40
Fe	1461.2	530.1	401.0	117	158	90
Cu	6.0	6.7	4.5	8.3	11.3	7.5
Zn	35.3	45.9	29.0	43	59	38
Ni	2.458	1.219	2.918			
Se	0.161	0.085	0.078			
Pb	0.591	1.108	0.641			

[a]The measurement was carried out 5 times. Data in the table are mean values of the measured data. Standard error of the mean (SEM) of each data was less than 1% of the mean value. SEM values are omitted for the clarity.
[b]Standard table of feed composition in Korea, National Institute of Animal Science, Korea (http://www.nias.go.kr/saryo/eng/animal.asp)

25.7% for *H. marmoreus*, *F. velutipes*, and *P. eryngii*, respectively (Table 4). On the contrary, mushrooms were not good at Ca uptake. Less than 0.4% of Ca in the cultivation substrate was taken by the fruiting bodies resulting in Ca concentrations of 159.8, 324.3, and 162.5 mg/kg dry weight in the fruiting bodies of *H. marmoreus*, *F. velutipes*, and *P. eryngii*, respectively.

Minor mineral elements in the cultivation substrates and the fruiting bodies. Fe is one of the important mineral elements for humans. The mushrooms in this study could take-up Fe to some extent (Table 3). *F. velutipes* was the best and accumulated 108.8 mg/kg in its fruiting body with a transfer ratio of 6.4%. Zn was present in fairly high amounts in all three mushrooms when compared with other trace elements. *P. eryngii* had the best accumulation of Cu. The concentration of Ni was intriguingly high in *H. marmoreus* even though Ni concentration in its cultivation substrate was lower than the others. *H. marmoreus* may have a special mechanism by which it can accumulate high amounts of Ni in its fruiting bodies. Recent evidence has shown that mushrooms can absorb metal ions in high concentrations (Bystrzejewska-Piotrowska *et al.*, 2008; Gonen Tasdemir *et al.*, 2008) and the metal absorption capability appeared to be species-specific (Alonso *et al.*, 2003). Therefore, it is very natural for different mushrooms to exhibit a preferential difference in absorbing mineral components. It is notable that metal binding peptides, namely phytochelatins which are found in most eukaryotic cells and some prokaryotes, preferentially form complex with transition metals such as Zn, Cu, and Ni (Clemens, 2006). Therefore, the phytochelatins in the fruiting bodies can be one explanation to address the high uptake capacity of mushrooms for Zn, Cu, and Ni. Se is recognized as an important micronutrient by exhibiting antioxidative protection effects against oxidative damage caused by free radicals and against development of certain types of cancer (Falandysz, 2008). The recommended daily intake of Se is 75 and 60 μg for men and women respectively (Barelay *et al.*, 1995). *H. marmoreus* and *P. eryngii* appeared to contain nutritionally significant but yet insufficient amounts of Se. Selenium enrichment in the cultivation substrate can be an approach to increase the Se concentration in fruiting bodies of mushrooms. The mycelia of *P. ostreatus* were enriched with Se when they were grown in Se rich medium (Serafin Muñoz *et al.*, 2006). Pb is toxic to humans. The legal limit concentration in food is 0.2 mg/kg (Korea Food and Drug Administration). Among three mushrooms, only *P. eryngii* contained insignificant amounts of Pb (0.002 mg/kg wet weight) while others were devoid of any.

Minerals in postharvest substrates. Mushroom spent cultivation substrates are mixtures of materials with high nutritional values including mushroom mycelia, degraded cellulosic fibers, degraded lignins, proteins, minerals etc., which make them a recognized valuable biological resource particularly as animal feed (Kim *et al.*, 2007a, b; Kwak *et al.*, 2008). In this regard, we assessed the micronutritional value of the spent substrates of *H. marmoreus*, *F. velutipes*, and *P. eryngii*. As shown in Table 5, the concentrations of the mineral components in the postharvest cultivation substrates are largely higher than those in the input cultivation substrates. This is probably due to the supply of mineral elements through moisture during the cultivation. In general, mushroom cultivation consists of two steps. The first is to propagate mycelia in the cultivation substrate which requires constant relative humidity (RH) around 65%. This step takes 25 days for *F. velutipes*, 35~40 days for *P. eryngii*, and 85~120 days for *H. marmoreus*. The second step is to grow fruiting bodies from the fully developed mycelia. It takes about a month of incubation with a relative humidity more than 85%. Therefore, it is most than likely that the elevated mineral contents in the postharvest cultivation substrates originated from the supplied water. In terms of micro-nutritional value, the mineral contents in the postharvest substrates were highly comparable with those in representative grains such as corn, barley, and wheat (Table 5).

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