Ciência Rural
ISSN: 0103-8478
cienciarural@mail.ufsm.br
Universidade Federal de Santa Maria
Brasil

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Ciência Rural, vol. 44, núm. 5, mayo, 2014, pp. 943-949
Universidade Federal de Santa Maria
Santa Maria, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=33130634029

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Ciência Rural, Santa Maria, v.44, n.5, p.943-949, mai, 2014
ISSN 0103-8478

Macro and trace elements in edible mushrooms, Shiitake, Shimeji and Cardoncello from Petrópolis, Rio de Janeiro, Brazil

Macro e elementos-traço em cogumelos comestíveis, Shiitake, Shimeji e Cardoncello, coletados em Petrópolis, Rio de Janeiro, Brasil

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ABSTRACT

*The concentrations of twenty-five elements (Al, As, Ba, Bi, Ca, Cd, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb , Sb, Se, Sn, Sr, V and Zn) were determined in three edible mushrooms, Shiitake (**Lentinula edodes)**, Black Shimeji (**Pleurotus ostreatus**) and Cardoncello (**Pleurotus eryngyii**) from Petrópolis, Rio de Janeiro, Brazil. Samples were collected along the year 2010 and their preparations were made after drying, milling, an acid pre-digestion and a decomposition procedure in a muffle furnace. The analytical techniques employed for the elements determination were Mass Spectrometry with Inductively Coupled Plasma and Flame Atomic Absorption Spectrometry. Two certified reference materials, Apple Leaves and Mussel Tissue, were used for the evaluation of the analytical procedure and recovery values around 98% were obtained. The results showed that the analyzed mushrooms have high levels of Cu, Fe, K, Mg, Mn and Zn containing more than 30% the recommended daily intake for these nutrients according to Brazilian legislation. These mushrooms presented a very low ratio Na/K. Regarding the levels of some contaminants, the mushrooms had concentrations of Cd, Pb and As below the recommended maximum limits allowed by Brazilian legislation.*

Key words: *edible mushroom, nutrients, contaminants*.

RESUMO

*As concentrações de vinte e cinco elementos (Al, As, Ba, Bi, Ca, Cd, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sn, Sr, V e Zn) foram determinadas em três cogumelos comestíveis, **Lentinula edodes** (Shiitake), **Pleurotus ostreatus** (Shimeji preto) e **Pleurotus eryngii** (Cardoncello) provenientes de Petrópolis, Rio de Janeiro, Brasil. As amostras foram coletadas durante o ano de 2010 e o preparo efetuado por secagem, trituração, pré-digestão ácida e mineralização em mufla. As técnicas analíticas utilizadas na determinação dos elementos foram a espectrometria de massa com plasma indutivamente acoplado e a absorção atômica com chama. Dois materiais de referência certificados, Apple Leaves e Mussel Tissue, foram utilizados para a avaliação do procedimento analítico e valores de recuperação em torno de 98 % foram obtidos. Os resultados demonstraram que os cogumelos estudados apresentam altos teores de Cu, Fe, K, Mg, Mn e Zn, contendo mais de 30% das quantidades recomendadas para ingestão diária desses nutrientes, conforme a legislação brasileira. Esses cogumelos possuem uma razão Na/K muito baixa e os níveis de alguns contaminantes, Cd, Pb e As, estão abaixo dos limites máximos permitidos pela legislação em vigor.*

Palavras-chave: *cogumelo comestível, nutrientes, contaminantes.*

INTRODUCTION

The use of mushrooms as source of nutrients or in therapeutics is old and crops for commercial purposes are described since sixth century. Commercial cultivation in Brazil is much more recent, initiated in the 50´s from the production of ***Agaricus bisporus***, commonly known as Champignon, in Mogi das Cruzes, São Paulo (BONONI, 2003).

Worldwide, the production of cultivated mushrooms is approximately 3.5 million metric tons, about 26 times lower than the production of meat that is about 88 million metric tons. In Brazil, *per capita* consumption of mushrooms is low, about 70 to 160

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Received 03.19.13 Approved 09.10.13 Returned by the author 03.19.14
CR-2013-0366.R2

grams habitant^{-1} year^{-1}, when compared with other countries like China, 10kg habitant^{-1} year^{-1} (KALAČ AND SVABODA, 2000). Just for comparison, *per capita* consumption of meat (beef and pork) in Brazil is 71kg year^{-1} while in China is 45 kg/year (FAO, 2003).

The species of mushrooms most widely cultivated and commercialized are "Champignon" which corresponds about 38% of the global market, followed by the species of the genus ***Pleurotus*** like ***Pleurotus ostreatus***, known as Shimeji, which correspond for about 25% of this market. ***Lentinula edodes***, known as Shiitake, appears in third place, which correspond around 10%. Brazil follows the order of cultivation and consumption of the species mentioned above, however "Champignon" is responsible for almost all domestic production which is about 3 metric tons/ year (VILELA, 2007).

Climate, culture factors and price limit the consumption of mushrooms in Brazil. The prevalence of tropical climate does not favor the growth of many edible species, the cultural pattern does not include mushrooms in regional recipes and in Brazil the mushrooms have high cost due to low productivity. Although these facts, the consumption has increased steadily since the last decade due to the adoption of habits coming from other regions of the globe, such as Japan, the inclusion of mushrooms as a sophisticated food and the adoption of public policies intended to train producers in mushroom cultivation (URBEN, 2004).

Under the nutritional aspect, mushrooms are a good source of protein, vitamins and inorganic elements like phosphorus, potassium, selenium, zinc, magnesium, copper (SADLER, 2003). MICHELOT et al. (1998) studied fifteen species from France, in the Paris region, reporting concentrations in the range of 340-1440 (Ca), 3-4 (Cd), 28-80 (Fe), 3-34 (Mn) 52-67 (Zn) mg kg^{-1} dw. ZHU et al (2003) investigated wild edible mushrooms from a Province of China and found concentrations ranged from 0.06 to12.9 (Cd, Pb and Ni) and 6.83 to 57.9 (Cu, Zn, Mn and Cr) and 95.7 to 242 (Fe) mg kg^{-1} dw. In a recent review from KALAČ (2010) the usual contents of four major elements reported from years 2000 to 2009 in European species are 100-400 (Na), 20000-40000 (K), 100-500 (Ca) and 800-1800 (Mg) mg kg^{-1} dw. In a complementary work, KALAČ (2010) reported trace elements concentrations in the range of 20-150 (Al), 0,5-5 (As), 2-4 (Ba), 1-5 (Cd), 0,5-5 (Cr), 20-100 (Cu), 25-200 (Zn) and 50-300 (Fe) mg kg^{-1} dw.

About the methods used for the determination of elements contents, MICHELOT and ZHU, used Inductively Coupled Plasma Optical Emission Spectrometry by (ICP-OES) but according to KALAČ (2010), Atomic Absorption Spectrometry (AAS) is the most wide-spread method. Nevertheless, there are reports about the use of another techniques: Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Anodic Stripping Voltametry (ASV) and Neutron Activation Analysis (NAA).

As pointed out by MOURA (2008), whom used the NAA technique, despite the existence of several works in others countries regarding the chemical composition of edible mushrooms, there are still few studies about their nutritional value. Therefore, the present study aimed to assess the nutritional importance of three species of mushrooms consumed in Brazil by evaluating the levels of inorganic trace elements.

MATERIALS AND METHODS

Samples of Shiitake, Shimeji and Cardoncello (***Pleurotus eryngyii***) were acquired at Cogumelos Imperial, a mushroom cultivation in the neighborhood of Rocio in Petropolis. The substrate used was composed by 80-90% residual of eucalyptus log (sawdust) and 10-20% of rice bran and wheat bran added of Gypsum or dolomite to obtain a pH 4. The substrate mixture used was dependent on the mushroom specie.

The samples were collected along the year of 2010, four samplings, and the amount collected was approximately 1 kilogram of each mushroom. The samples were washed and after drying in oven at 50°C during 4 hours, they were packaged with a low density polyethylene plastic envelops and then taken to the laboratory where they were stored in refrigerator under 7ºC overnight.

To determine the moisture content of mushrooms, approximately 5g of each fresh mushroom (n=5) were weighed, oven dried at 90ºC during 2 hours. Afterwards they were kept in a desiccator until reach room temperature. The procedure was performed twice.

To proceed the analysis, the samples were dried in oven at 90°C for 2 hours, grounded in domestic processor and packed in polypropylene plastic bottles. After drying, a pre-digestion of ten aliquots of 0.5g from each mushroom collected along the year of 2010 were performed with concentrated HNO_3 (Suprapur Merck) and heated in a hot plate for 4h. Than, the samples were ashed during 12 hours in an oven at 500ºC. The ashes were dissolved with 10mL of 10% w/v and transfer with deionized water (Milli-Q Millipore 18.2MΩ cm^{-1} resistivity) for polypropylene bottles in a final volume of 15mL. Blank solutions of the reagents and replicates (n=5) of certified reference materials, Apple Leaves NIST n.1515 and Mussel Tissue from National Institute of Standards and Technology (NIST) n.2976, were made in each digestion procedure to ensure the quality of results. The quantification of inorganic elements was performed with Inductively Coupled Plasma Mass Spectrometer (ICP–MS) model 2 X-Series – Thermo Scientific and a Flame Atomic Absorption Spectrometer (FAAS), model AA400, Perkin Elmer.

RESULTS AND DISCUSSION

The performance of the analytical procedure was evaluated by analyzing the certified reference material (RCM) and for most elements were found concentrations consistent with expected values, they were between 88-102% of certified values. The relative standard deviation of the method was evaluated by analyzing five individual aliquots from the two RCM and for all elements analyzed it was between 2 and 11%.

There were no significant variations in elements concentrations for all mushroom species. It could be attributed to similar environmental conditions of cultivation and substrate composition, even so, some variability was observed. The small variations between mushrooms species were not constant and this may perhaps due to the size and weight of mushrooms collected, factors that were not considered in this study.

The average moisture content of studied species was approximately 90%: Shiitake, 90,8±1,1; Shimeji, 90,1±1,4 and Cardoncello, 88,9±0,9. The average levels of the elements in each mushroom are on a dry weight basis and summarized in tables 1 and 2. Shimeji presented highest concentrations of Ba, Ni, As, Bi, Cr, Ca, Ga, Mo, Sb, Se, Sr, Sn, V and K, followed by Shiitake with higher levels of Cd, Cr, Pb, Sn, Mn, Zn, Na and Mg, and Cardoncello with higher levels of Li, V, Fe, Rb, Ca and Al. The results for Shiitake samples were discordant for the following elements: Ga, Mo and Sn. The 3rd sampling had lower values for Ga - 0.087mg kg^{-1} dw, higher values in the 4th collection for Mo - 1.24mg kg^{-1} dw, and higher values in the second collection for Sn - 0.206mg kg^{-1} dw.

The results showed the macro and micronutrients with higher variability concentration among the four sampling were: Zn - 74, 134, 82, 158mg kg^{-1} dw and Cu - 2.3, 1.7, 12.5, 7.4mg kg^{-1} dw. Shimeji species presented variations only for two elements: Se - 0.078, 0.16, 0.123, 0.040, and Al - 51, 115, 114, 50mg kg^{-1} dw.

Each species of mushroom has been classified by comparing the amount of nutrients with the value of the Recommended Daily Intake (RDI) according to Brazilian legislation (BRASIL, 2005) in which a food is considered a nutrient source when a minimum of 15% of RDI in 100g is reached and a high level source of nutrient with at least 30% of RDI in 100g. In agreement with this legislation,

Table 1 – Micronutrients and trace-elements concentration in Shiitake, Shimeji and Cardoncello mushrooms.

Mushroom	Concentration (dry weight) mg kg^{-1}																				
	Al	Ba	Cu	Fe	Li	Mn	Ni	Rb	Zn	As	Bi	Cd	Cr	Ga	Mo	Pb	Sb	Se	Sn	Sr	V
Shiitake																					
Mean	82	1.4	6.1	52	0.8	52	1.5	48	112	0.14	0.034	0.18	0.64	0.16	0.10	0.26	0.033	0.030	0.10	0.031	0.09
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
SD	32	0.1	2	11	0.3	19	0.1	7	37	0.04	0.003	0.05	0.11	0.08	0.02	0.04	0.003	0.008	0.04	0.004	0.02
Shimeji																					
Mean	83	1.9	21	76	1.5	24	4.2	88	65	0.17	0.038	0.11	0.75	0.20	0.08	0.17	0.038	0.10	0.09	0.039	0.12
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
SD	40	0.4	6	13	0.6	7	0.3	9	11	0.01	0.002	0.01	0.03	0.02	0.01	0.02	0.004	0.05	0.03	0.004	0.02
Cardoncello																					
Mean	93	1.8	9.4	102	1.5	22	3.6	94	99	0.017	0.036	0.11	0.025	0.09	0.17	0.18	0.031	0.10	0.05	0.013	0.21
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
SD	79	0.2	1.1	26	0.1	9	0.1	29	23	0.001	0.002	0.04	0.003	0.04	0.04	0.10	0.004	0.02	0.01	0.002	0.07

Note: Mean and standard deviation (SD) values from four sampling period.

Table 2 – Macronutrients concentration in Shiitake, Shimeji and Cardoncello mushrooms.

Mushroom	------------Concentration (dry weight)------------- ------------------------mg kg^{-1}-----------------------			
	Ca	Na	K	Mg
Shiitake				
Mean	47	101	15000	4560
	±	±	±	±
SD	4	10	1654	320
Shimeji				
Mean	55	50	24585	2410
	±	±	±	±
SD	6	2	3741	212
Cardoncello				
Mean	59	65	17540	3483
	±	±	±	±
SD	3	8	1205	495

Note: Mean and standard deviation (SD) values from four sampling period.

all three species of mushrooms were characterized as food with high levels of Cu, K, Mg, Mn and Zn (Table 3). Nevertheless, considering that all levels are expressed in dry weight and the average moisture of studied species (90%), fresh mushrooms content of minerals is relevant but the mushrooms could not be classified as major source of the analyzed elements.

Sodium mean concentration for Shimeji was 46mg kg^{-1} dw, a concentration lower than 50mg kg^{-1} dw, for that reason it can be classified as a food that contains no Na according to Brazilian legislation (BRASIL, 1998). Shiitake (101mg kg^{-1} dw) and Cardoncello (65mg kg^{-1} dw) presented Na mean concentrations higher than 5mg $100g^{-1}$ and can be classified as food with low Na concentrations. However, K concentration in the three mushrooms species analyzed was high in the range of 14000-32500mg kg^{-1} dw, which allows classified them as foods with high potassium content.

According to the results, Na/K concentrations ratio in all mushrooms species were very low. The values for Shimeji - 0.001871<Cardoncello - 0.0037058<Shiitake - 0.0067333 were much lower than ratios of 1.11 to 1.92 which according to YANG et al. (2011) are associated with a significantly increased risk of death from cardiovascular disease in the U.S. population.

The mean Fe concentrations for Shiitake, Shimeji and Cardoncello were 52, 76 and 102mg kg^{-1} dw, respectively. These results were in agreement with previous studies: 13 and 33mg kg^{-1} dw for Shiitake (DE PAULI, 2010 and ÇAĞLARIRMAK, 2007), 15, 54 and 682mg kg^{-1} dw for Shimeji (ÇAĞLARIRMAK, 2007; MATTILA, 2001; GENÇCLEP et al, 2009) 31 and 355mg kg^{-1} dw for Cardoncello (MOURA, 2008; GENÇCLEP et al., 2009).

The Mo concentration represented 18-38% of RDI value (Table 3) and according to some studies adequate amounts of Mo are important for the prevention of esophageal cancer (PHILIP et al., 1994;

Table 3 – Comparison between the contents of total elements in the three species of mushroom with the recommended values[3] for daily intake (RDI).

----------------RDI – mg----------------		------------------------------------Concentration mg 100 g^{-1} (dry weight)-------------------------------------				
	Adults	Shiitake	Shimeji	Cardoncello	Lower conc.	% RDI
Ca[1]	1000	4.7	5.5	5.9	4.7	0.47
Cr[1]	0.035	0.064	0.075	0.0025	0.0025	7.14
Cu[1]	0.9	0.6	2.1	0.9	0.6	66.67
Fe[1]	14	5.2	7.6	10.2	5.2	37.14
Mg[1]	260	456	241	348	241	92.69
Mn[1]	2.3	5.2	2.4	2.2	2.2	95.65
Mo[1]	0.045	0.010	0.008	0.017	0.008	17.78
Se[1]	0.034	0.003	0.010	0.010	0.003	8.82
Zn[1]	7	11.2	6.5	9.9	6.5	92.86
K[2]	3500	1500	2458	1754	1500	42.86
Na[2]	2400	10.1	5.0	6.5	5.0	0.21

Notes:
1- BRASIL. Resolution nº 269, 2005.
2- FAO 2013.
3- % RDI was calculated using the RDI values and the lower concentration of the element found in mushrooms.

Table 4 – Maximum acceptable limits (MAL), provisional tolerable weekly intake (PTWI) and element contents in mushrooms.

Element	MAL[1] mg kg^{-1}	PTWI[2] mg kg^{-1} body weight (x 70kg)*	Mean values (mg kg^{-1} dry weight)		
			Shiitake	Shimeji	Cardoncello
Al		1 (70)	80	83	93
Sb	2		0.003	0.004	0.003
As	1	0.015 (1.05)	0.014	0.016	0.002
Cd	1	0.007 (0.49)	0.018	0.010	0.011
Pb	0.8	0.025 (1.75)	0.026	0.017	0.018
Cu	30	3.5 (245)	0.6	2	0.9
Cr	0.1		0.31	0.36	0.18
Sn	250	14 (980)	0.007	0.009	0.005
Fe		0.8 (56)	41	76	102
Ni	5		1.5	4	3.6
Se	0.3		0.003	0.007	0.009
Zn	50		11	6.5	10

Notes:
[1]Brasil, 1965.
JECFA Reports. *Estimated body weight of an adult male.

MOHSEN et al., 2008). Mo is a cofactor of enzymes such as nitrogenase, nitrate reductase, sulphite oxidase and xanthine oxiredutase (SCHWARZ et al., 2009). Although mushrooms are consumed in small quantities in Brazil, the Mo content is a relevant composition characteristic from the studied species.

In other studies with the same mushroom species (DEMIRBAS, 2001; MATILLA, 2001; MOURA, 2008; WANG, 2001; MANZI et al., 1999; GENÇCLEP et al., 2009) the mean levels of most elements were consistent with those in the present study. It should be emphasized that different substrate composition used in each crop results in different levels of elements as proven by FIGUEIRÓ (2011) who worked with six different substrates (Straw of rice, beans, wheat, sorghum, banana leaves and corn cob). They reported a mean Fe concentration of 96mg kg^{-1} in rice straw substrate and 476mg kg^{-1} in banana leaves; a mean Mn concentration of 7.8mg kg^{-1} in corn cob and 835mg kg^{-1} in banana leaves and for Zn a mean concentration of 7.5mg kg^{-1} in a banana leaves and 30.2mg kg^{-1} in rice straw.

No published data were found about Al, Ba, Bi, Li, Ni, Sr and Sb in Shiitake and Cardoncello and the same for Mo, Sn and V in the three mushroom species studied.

The mean concentration of Sb, As, Cd, Pb, Cu, Cr, Sn, Fe, Ni, Se and Zn were compared with the maximum limits of tolerance for metals in mushroons or vegetables foodstuffs (Table 4) established by Brazilian legislation (BRASIL, 1965) and in general, metal concentrations were below these recommended values.

There are no recommended maximum limits of Al in foods in Brazil, however, significant results were observed for Al in mushrooms: 82 and 83mg kg^{-1} dw in Shitake and Shimeji and 93mg kg^{-1} dw in Cardoncello. These values were very close to the recommended weekly intake by JECFA (Joint FAO/ WHO Expert Committee on Food Additives) 70mg kg^{-1} of an adult male, and could represent a risk depending on the amount of ingested mushroom.

CONCLUSION

All mushrooms studied can be considered as foods with high content of Cu, K, Mg, Mn and Zn if they are consumed dry and they have a very low Na/K ratio. The samples do not present a health risk with Pb, Cd and As once the levels presented lower than maximum limits recommended by Brazilian legislation. Since 1996 the CODEX ALIMENTARIUS, Commission of World Health Organization and Food and Agriculture Organization considered that there are a moderate data about Al in food. The authors endorse the importance to develop programs including the determination of this metal in several food products.

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