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Major, Minor and Toxic Minerals and Anti-Nutrients Composition in Edible Mushrooms Collected from Ethiopia

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Abstract

Major (Na, K, Ca, Mg, P), minor (Mn, Cu, Fe, Zn) and toxic (Pb, Cd) minerals composition of twelve edible mushrooms (*Pleurotus ostreatus, Lentinula edodes, Agaricus bisporus #1 (fresh), Agaricus bisporus #2 (canned), Agaricus campestris, Laetiporus sulphureus, Termitomyces clypeatus, Termitomyces microcarpus #1, Termitomyces microcarpus #2, Termitomyces aurantiacus, Termitomyces letestui* and *Termitomyces species*) collected from three regions of Ethiopia were analyzed. The samples were further investigated for their antinutrients (phytate and condensed tannin) to determine bioavailability of minerals. All the results are expressed in dry basis (db). The major minerals concentration (mg/g) ranged: Na (0.41-34.8), K (3.66-42.4), Ca (0.29-6.45), Mg (0.57-2.12) and P (0.71-2.82). The minor (mg/kg) ranged: Fe (32.5-6835.9), Zn (26.6-87.6), Cu (5.69-45.9) and Mn (0.96-138.6). The toxic metal lead was detected (1.52-18.0 mg/kg), indicating most of the mushrooms samples exceeded the weekly tolerance limit set for Pb with more proportion in wild than cultivated mushrooms. Cadmium was detected only in A.campestris (4.08 mg/kg). The anti-nutrients (mg/100 g) were significantly varied with phytate ranged from 31.3 to 242.8 and condensed tannin from 4.81 to 31.7. The calculated molar ratio between phytate and Fe, Zn and Ca was above the suggested critical values indicating the bioavailability of Fe, Zn and Ca to be high. In conclusion, the results imply that the edible mushrooms have high concentrations of essential minerals with lower anti-nutrients that make them bioavailable to the human body. Although, the consumption of some contaminated mushrooms should be avoided.

Keywords: Mushroom; Minerals; Major; Minor; Toxic; Anti-nutrients; Bioavailability

Introduction

The wide variety and abundance of minerals is the most valuable part of mushrooms as food which is related to species of mushroom, collecting area of the sample, age of fruiting bodies and mycelium, and distance from any source of pollution [1]. Potassium, phosphorous, copper, cobalt, aluminum and zinc are often present in mushrooms in high quantities. Some mushrooms tend to collect any minerals from their surroundings, even the toxic heavy metals such as lead, cadmium and mercury that are present in polluted areas [2].

It is also important to consider anti-nutritional factors that may be present in raw mushrooms. For example, it is known that *A.bisporus* and *P.ostreatus* contain hemagglutinins that have shown to interfere with protein absorption in rats and have even produced lesions in the small intestine [3]. Moreover, mushrooms have been shown to contain some secondary plant products such as phytates, alkaloids, saponins, tannins and oxalate [4], which may decrease the bioavailability of the minerals. Bioavailability is a general term that refers to how well a nutrient can be absorbed and used by the body. It can be affected by many factors such as the presence of anti-nutrients, for example, phytates, oxalates, tannins and polyphenols in foods, a person's need, fibre, competition with other nutrients and acidity of intestinal environment [5].

Phytic acid, a compound found only in plant foods [6] is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. The presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phytate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption [7].

Condensed tannins (or proanthocyanidins, PAs) comprise a group of polyhydroxy-flavan-3-ol oligomers and polymers linked by carbon-carbon subunits [8]. The reactivity of PAs with molecules of biological significance had important nutritional and biological consequences. Their multiple phenolic hydroxyl groups lead to the formation of complexes with proteins [9-11] with metal ions [12-13] and with other macromolecules like polysaccharides [14].

There are many techniques used to determine the bioavailability of minerals in the human body. One of the methods is by measuring the molar ratio of phytate/minerals in the food and diet [15]. The proportion of samples with ratios above the suggested critical values have been calculated: [phytate]: [calcium] >0.24 [16], [phytate]: [iron] >1 [17], [phytate]: [zinc] >15[15,18,19] [phytate]* [calcium]/[zinc] > 0.5 mol/kg [20,21].

Studies investigating the significance of the molar ratios of [Phy]: [Fe], [Phy]: [Zn], [Phy]: [Ca], [Phy] *[Ca]: [Zn] and proportion of the phytate phosphorus and non phytate phosphorus for determining of these minerals status obtained from edible mushrooms are not as exhaustive as studies done on cereals and legumes. Kalač [22] reported digestibility and bioavailability of mushroom constituents have been missing from the knowledge of mushroom nutritional value. Few authors such as Aletor [23], Ola et al., [24], Akindahunsi et al., [25] reported the concentration of antinutritional factors of some edible

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mushrooms. In addition, no information is available about either the minerals or anti-nutrients composition of Ethiopian mushrooms.

Consequently, in this study the concentration of major minerals (Na, K, Ca, Mg, and P), minor minerals (Zn, Fe, Cu, and Mn) and toxic minerals (Pb, Cd) and the antinutrients (phytate and condensed tannin) of edible mushrooms collected from Ethiopia were evaluated. Accordingly, the respective [Phy]: [Fe], [Phy]: [Zn], [Phy]: [Ca], [Phy]*[Ca]: [Zn] molar ratios and the proportion of phytate phosphorus and non phytate phosphorus were calculated and compared to the critical values to predict the bioavailability of Fe, Zn, Ca and P obtained from these edible mushrooms to the human body.

Materials and Methods

Description of sampling areas

The three mushroom sampling areas were Addis Ababa, Kaffa zone and Benishangul Gumuz region of Ethiopia. Addis Ababa is the capital city of Ethiopia and located 9°01 N and 038°45 E. Kaffa zone is situated in the northwestern part of the southern nations, nationalities and people region state (SNNPR) and lies within 07° 00'-7°25'N latitude and 35°55'-36°37'E Longitude. Benishangul gumuz region is located in western parts of Ethiopia located between 09.17°-12.06° North latitude and 34.10°-37.04° East longitude.

Sample collection and identification

The collection of samples was based on their abundance and availability during the rainy season of the year (May-September). The samples were either collected from the field or purchased from the indigenous people who collect edible forest resources in the region or from the local markets. Identification of the wild edible mushrooms was made by making comparisons with authentic illustrations. Moreover, confirmations of the wild mushrooms were made by mycological experts at the department of life sciences at Addis Ababa University.

Within a two consecutive year a total of 12 edible mushrooms were collected where four of them were cultivated and eight of them were wild mushrooms. These are *Pleurotus ostreatus, Lentinula edodes, Agaricus bisporus #1 (fresh), Agaricus bisporus #2 (canned), Agaricus campestris, Laetiporus sulphureus, Termitomyces clypeatus, Termitomyces microcarpus #1, Termitomyces microcarpus #2, Termitomyces aurantiacus, Termitomyces letestui* and unidentified *Termitomyces* species. The first five mushrooms are collected from Addis Ababa, the next three are from Kaffa and the last four are from Benishangul Gumuz region, respectively.

Preparation of samples and storage

The mushroom samples were cleaned out of forest debris (without washing) with a plastic knife and sliced without separating the cap and the stipe of the mushrooms. Samples were dried in drying oven in the laboratory till constant weight. The dried samples were milled to fine powder (20 mesh) using a mill (FW 100, Yusung Industrial Ltd, China) and kept in plastic bottles until analysis.

Determination of minerals (major, minor and toxic)

Prevention of contamination: To minimize the risk of contamination, glass wares were washed with 10% HNO_3 acid and crucibles were soaked with 6N HCl for 24 hours after being washed with detergent and water. All materials were then rinsed with distilled-deionized water and dried in an oven before use.

Method detection limit and calibration curve: Estimation of the mineral concentration of the blank is important for the determination of the detection limit of the analytical method. Thus, eight reagent blanks were prepared in parallel and analyzed for their metal content as the samples. The pooled standard deviation of the eight blanks was calculated and multiplied by three to determine the method detection limit (MDL) of each metal [26]. Sample concentrations which are below the method detection limit (µg/g) were reported as not detected (nd). Calibration curve for each mineral was constructed using an appropriate standard at a series of concentrations. Regression equation for each metal standard was constructed and best fit of the equation was checked using correlation coefficient (R^2).

Digestion of mushroom samples: Digestion of mushroom samples was carried out by following the dry ashing method [27,28] with some modifications. Two grams of sample was placed in a porcelain crucible and ashed in an oven at 450°C for 24 h. Ashed material was dissolved in 2 ml of concentrated HNO_3, evaporated to dryness, heated again to 450°C for 4 h. Samples then was dissolved in 2 ml of concentrated H_2SO_4, 2 ml concentrated HNO_3 and 2 ml of H_2O_2, and diluted with distilled-deionized water up to 100 ml after adding 1 % $LaCl_3$ (2 ml) as releasing agent for Ca and Mg minerals. A blank digest was carried out in the same way.

Analysis of minerals with atomic absorption spectrometer: For the mineral analyses, PG-990 atomic absorption spectrometer (Beijing, China) was used by setting the appropriate wavelength for each metal. All metal ions (expect K, Na and P) determination was carried out in an air/acetylene flame mode of the spectrophotometer. K and Na levels in the mushroom samples were determined by CL 378 flame photometer (ELICO Limited, UK) using butane gas. Phosphorus was determined by UV-VIS spectrophotometer (Perkin Elmer, UK) using the molybdovanadate method [29].

Quality control: The reliability of the measurements was assessed by analyzing some of the minerals using the certified reference materials IRMM 804 (rice flour) and BCR-381 (Rye Flour) supplied by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). For assessing the method performance, the measured values of a CRM were compared with the certified values. Moreover, a control sample was digested and analyzed with each analytical batch of samples to check the effectiveness of the digestion procedure. In order to further control the stability of the measurements, the last concentration of the standard solution was placed in a certain location of the auto-sampler loading list and it was analyzed every 10 samples.

Antinutritional factors and bioavailability of minerals

Phytate: The phytate content in the sample was determined according to the method described by Latta et al. [30] and later modified by Vaintraub et al., [31]. About 0.1 g of fresh samples was extracted with 10 ml 2.4% HCl in a mechanical shaker for 1 hour at a room temperature. The extract was centrifuged at 3000 rpm for 30 minute. The clear supernatant was used for phytate estimation. One ml of wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalcilic acid in water) was added to 3 ml of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 seconds. Absorption readings at 500 nm were taken against a blank sample consisting of 3 ml extract solution with 2 ml of 2.4% HCl without wade reagent. Sodium salt of phytic acid (4.5-36 mg/ml) was used as standard for construction of calibration curve (Absorbance=-0.0064 phytic acid mg + 0.3655, R^2= 0.9916).

Condensed tannin: Tannin was determined by Burns et al.,[32]

as modified by Maxon ED [33]. One gram of sample was weighed and mixed with 10 ml 1% HCl in methanol in a screw cap test tube. Then the tube was shaken for 24 hr at room temperature on a mechanical shaker. The solution was centrifuged at 1000 rpm for 5 minutes. One ml of supernatant was transferred to another test tube and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). After 20 minutes, the absorbance of the solutions and the standard solution were measured at 500 nm. Blank sample consisted 1 ml of extract solution with 5 ml of 1% HCl without vanillin-HCl reagent. (+) catechin (0.5-12 mg /100 ml) was used as standard for construction of calibration curve (Absorbance=0.0054 (+) -catechin mg + 0.0024, R^2=0.9971).

Determination of molar ratio of phytate/mineral: The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660 g/mol; Fe: 56 g/mol; Zn: 65 g/mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals [34]. Phytate phosphorous was calculated by assuming phytate contains 28% phosphorus, i.e. [Phytate P=phytate *0.28] and accordingly non phytate phosphorous=total phosphorous-phytate phosphorous.

Statistical analysis

Completely randomized design (CRD) was used. All the experimental results were reported as mean ± standard error (SE) of three parallel measurements. Data were evaluated by using one way variance analysis (ANOVA) and means were separated by Duncan' multiple range test (DMRT).A linear regression correlations test (R^2) was performed to investigate correlations between metal concentrations. SPSS version 15.0 was used for determining of significance at $p<0.05$.

Results and Discussion

Method detection limits and quality assurance

The method detection limits, wavelengths at which analysis was done and the correlation coefficients of the calibration curve for each of the metals. The results clearly show that method detection limits and the calibration curves were in good range for the analysis of metals in the mushroom samples. Moreover, the measured values of the certified reference materials are somewhat in good agreement with the certified values.

Major minerals

As shown in Table 1 the concentration of the major minerals in dry basis (Na, K, Ca, Mg and P) is expressed in mg/g. The sodium content ranged from 0.41 mg/g (*T.letestui*) to 34.8 mg/g (*A.bisporus*#2). The higher amount of sodium in *A.bisporus*#2 is due to the incorporation of salt in addition to citric acid and water during canning of whole or sliced button mushrooms. The sodium concentration in mushroom from other report ranges from 0.03-4.85 mg/g [35]. Thus, excluding the canned mushroom (i.e. *A.bisporus*#2), the sodium content of Ethiopian mushrooms were in the range of 0.41 to 1.83 mg/g which is in good agreement with the literature. The concentration of sodium is relatively low (except canned mushroom) and this is of very great nutritional benefit to the consumer, a finding that has been corroborated by Vetter [36]. Potassium content was higher than other minerals in all mushrooms in this study, varying between 3.66 mg/g (*L.sulphureus*) and 42.4 mg/g (*T.clypeatus*). Potassium concentration in mushrooms from other report ranges from 12.6 to 29.1 mg/g dry basis. The low concentration of sodium and the presence of a great amount of potassium suggest the utilization of mushrooms in an anti-hypertensive diet (Table 1).

Calcium content ranged from 0.29 mg/g (T.sp) to to 6.45 mg/g (*T.aurantiacus*) indicating these mushrooms could be a good source of calcium. Calcium content in mushrooms from other report ranges from 0.17 to 8.80 mg/g dw [35]. Magnesium varied from 0.57 mg/g (*L.sulphureus*) to 2.12 mg/g (*P.ostreatus*) again indicating these mushrooms could be a good source of minerals. Magnesium content in mushrooms from other report ranges from 0.90 to 4.54 mg/g dw [35]. Phosphorus varied from 0.71 mg/g (*A.bisporus*#2) to 2.82 mg/g (*A.bisporus*#1). Phosphorus content in mushrooms from other report ranges from 0.64 to 4.49 mg/g dw [35]. As compared with vegetables, mushrooms proved to be good sources of various minerals with K and P being the main constituents of mushrooms ash [37].

Minor (trace) minerals

Trace minerals are of great biochemical interest and exhibit nutritional and clinical importance [38]. The concentrations of trace minerals in dry basis (Fe, Zn, Cu, and Mn) in the edible mushroom species based on dry weight as mg/kg were shown in Table 2. The lowest iron content was 32.5 mg/kg for *A.bisporus*#2 and the highest iron content was observed by *T.microcarpus*#1 with 6835.9 mg/kg. Iron concentration of mushrooms was reported in the ranges of 180 to 407 mg/kg [39], 7.5 to 142 mg/kg [40], 50.1-842 mg/kg [35], 31.3-1190 µg/g [41], 568-3904 µg/g [42], 56.1-7162 µg/g [43]. 102-1580 µg/g [44] 30-

No.	Mushroom Type	Na	K	Ca	Mg	P
1	*P.ostreatus*	0.83 ± 0.17c,d,e	20.6 ± 0.15c,d	1.16 ± 0.06d	2.12 ± 0.02a	2.50 ± 0.03b,c
2	*L.edodes*	0.99 ± 0.00c,d,e	14.3 ± 0.17e	2.07 ± 0.12b,c	1.65 ± 0.05c	1.16 ± 0.02f
3	*A.bisporus#1*	0.77 ± 0.02d,e,f	23.7 ± 0.11c	2.24 ± 0.26b	1.92 ± 0.03b	2.82 ± 0.03a
4	*A. bisporus#2*	34.8 ± 0.12a	6.46 ± 0.02f	1.69 ± 0.24c	0.90 ± 0.04f	0.71 ± 0.03g
5	*A.campestris*	1.17 ± 0.17c,d	17.3 ± 0.60d,e	0.91 ± 0.08d,e	1.57 ± 0.04c	2.14 ± 0.11d,e
6	*L.sulphureus*	0.67 ± 0.17e,f	3.66 ± 0.16f	0.80 ± 0.08d,e	0.57 ± 0.00h	1.34 ± 0.04f
7	*T.clypeatus*	1.83 ± 0.44b	42.4 ± 0.28a	0.94 ± 0.08d,e	2.00 ± 0.01a,b	2.65 ± 0.03a,b
8	*T.microcarpus#1*	1.75 ± 0.14b	32.6 ± 3.42b	0.83 ± 0.18d,e	1.10 ± 0.11e	2.16 ± 0.21c
9	*T.aurantiacus*	0.49 ± 0.00e,f	22.1 ± 1.30c	6.45 ± 0.20a	1.30 ± 0.08d	1.36 ± 0.07f
10	*T. microcarpus #2*	1.33 ± 0.29b,c	29.5 ± 2.07b	0.89 ± 0.09d,e	1.06 ± 0.06e	1.98 ± 0.11e
11	*T.letestui*	0.41 ± 0.04f	22.8 ± 1.64c	0.55 ± 0.09e,f	0.73 ± 0.04g	2.08 ± 0.06e
12	*T. sp*	0.43 ± 0.01f	24.4 ± 0.01c	0.29 ± 0.01f	0.88 ± 0.03f,g	2.36 ± 0.05c,d

- Means followed by same letter in the same column are not significantly different ($p>0.05$)
- Data are mean ± standard error of three parallel measurements (n=3)

Table 1: Major minerals content (mg/g) of cultivated and wild edible mushrooms collected from Ethiopia in dry basis.

No.	Mushroom Type	Fe	Zn	Cu	Mn
1	*P.ostreatus*	72.7 ± 3.49e	61.8 ± 1.16d	11.1 ± 0.58g,h	1.72 ± 0.15f
2	*L.edodes*	98.0 ± 1.29e	80.9 ± 0.94b	12.2 ± 0.73g	10.8 ± 0.40d,e,f
3	*A.bisporus#1*	63.2 ± 0.05e	81.5 ± 0.28b	39.0 ± 0.46b	2.95 ± 0.10f
4	*A. bisporus#2*	32.5 ± 0.92e	26.6 ± 1.74g	8.59 ± 0.29h	1.46 ± 0.36f
5	*A.campestris*	1679.5 ± 23.94c	87.6 ± 0.89a	33.6 ± 0.96c	59.2 ± 1.07b
6	*L.sulphureus*	168.9 ± 11.1e	38.8 ± 1.06f	5.69 ± 0.77i	0.96 ± 0.01f
7	*T.clypeatus*	406.8 ± 7.70d,e	76.9 ± 0.54c	45.9 ± 0.43a	13.4 ± 0.31d,e
8	*T.microcarpus#1*	6835.9 ± 1925a	61.3 ± 8.06d	10.9 ± 1.78g,h	138.6 ± 10.6a
9	*T.aurantiacus*	1378.4 ± 114c,d	44.0 ± 1.69e	14.8 ± 0.82f	3.73 ± 0.74e,f
10	*T. microcarpus #2*	3192.4 ± 336.8b	84.4 ± 4.83a,b	8.78 ± 0.57h	25.7 ± 2.75c
11	*T.letestui*	1265.7 ± 55.6c,d	74.9 ± 0.56c	20.5 ± 0.74e	19.9 ± 0.64c,d
12	*T. sp*	6517.5 ± 83.9a	74.3 ± 0.33c	23.9 ± 0.89d	64.2 ± 3.08b

- Means followed by same letter in the same column are not significantly different (p>0.05)
- Data are mean ± standard error of three parallel measurements (n=3)

Table 2: Minor minerals content (mg/kg) of cultivated and wild edible mushrooms collected from Ethiopia in dry basis.

150 µg/g [2], respectively. Thus, as compared to other studies, the iron content of Ethiopian mushrooms was in agreement with some of the reported values in the literature. It is known that adequate iron in a diet is very important in order to decrease the incidence of anemia (Table 2).

As indicated in Table 2, the zinc content was the lowest (26.6 mg/kg) in *A.bisporus* #2, whereas the highest (87.6 mg/kg) in *A.campestris*. Mushrooms are known as zinc accumulators and the sporophore: substrate ratio for Zn ranges from 1 to 10 mg/kg [43] Zinc is widespread among living organisms, due to its biological significance. A zinc concentration of mushrooms was reported in the ranges of 30–150 µg/g [2], 33.5– 89.5 µg/g [44] and 29.3–158 µg/g [43]. Hence, zinc content in mushrooms of the present study is in agreement with previous studies [2,28].

The minimum and maximum values of copper were 5.69 and 45.9 mg/kg in *L.sulphureus* and *T.clypeatus*, respectively. Copper content of mushroom samples in other reports ranged from 4.71–51.0 µg/g [45] and 10.3–145 µg/g [41]. Similar to the reports copper content of Ethiopian mushroom species are within range and can be a good source of copper. The values, such as 45.9 mg/kg for *T.clypeatus*, 39.0 mg/kg for *A. bisporus*#1 and 33.6 mg/kg for *A.campestris* are high. This indicates consuming 30 g of dry matter of these three mushrooms a person can meet the recommended dietary allowance (RDA) of 900 µg/day set by EU [2,46] reported that Cu levels in the accumulating species are usually 100–300 mg/kg dry matter, which is not considered a health risk. Copper content in mushrooms is higher than those in vegetables should be considered as a nutritional source of the element [35].

The manganese content of the mushrooms ranged from 0.96 mg/kg (*L.sulphureus*) to 138.6 mg/kg (*T.microcarpus*#1). The values of manganese in the literatures for mushrooms were 14.2–69.7 µg/g, 21.7–74.3 µg/g, 7.1-81.3 ug/g and 5.54-135 mg/kg respectively [28,35,44,47]. The manganese values in this study are in agreement with results in the literature.

One important observation in this study was the only wood inhabiting polypores (*L.sulphureus*) evaluated in this study had the least mineral (Ca, Mn, Cu, Mg, K) concentration [48] reported that the members of the Polyporaceae (polypores) had relatively low mineral contents, and the percentage of copper, zinc, manganese and lead was lower in mushrooms that grew on wood than on the ground.

Toxic minerals

Many wild edible mushroom species are known to accumulate high levels of heavy (toxic) metals and mainly cadmium, mercury

No.	Mushroom Type	Cd	Pb
1	*P.ostreatus*	nd (< 0.028)	5.45 ± 1.09c
2	*L.edodes*	nd (< 0.028)	1.52 ± 0.73d
3	*A.bisporus#1*	nd (< 0.028)	18.0 ± 0.11a
4	*A. bisporus#2*	nd (< 0.028)	11.7 ± 0.73b
5	*A.campestris*	4.08 ± 0.38a	8.68 ± 1.95b
6	*L.sulphureus*	nd (< 0.028)	3.40 ± 0.88c,d
7	*T.clypeatus*	nd (< 0.028)	10.1 ± 1.19b
8	*T.microcarpus#1*	nd (< 0.028)	4.03 ±0.30c,d
9	*T.aurantiacus*	nd (< 0.028)	9.84 ± 1.66b
10	*T. microcarpus #2*	nd (< 0.028)	12.3 ± 0.32b
11	*T.letestui*	nd (< 0.028)	17.2 ± 1.51a
12	*T. sp*	nd (< 0.028)	11.9 ± 1.72b

- Means followed by same letter in the same column are not significantly different (p> 0.05)
- Data are mean ± standard error of three parallel measurements (n=3)

Table 3: Toxic minerals content (mg/kg) of cultivated and wild edible mushrooms collected from Ethiopia in dry basis.

and lead [2], perhaps related to the environment in which they are picked [49]. The two toxic metals cadmium and lead were evaluated here for Ethiopian mushrooms. Cadmium was detected only in one of the sample (*A.campestris*) at a concentration of 4.08 mg/kg in dry basis (Table 3), while the others are below the MDL (<0.03 µg/g). This exceptionality of *A.campestris* might be due to the proximity of the samples to roads [50] reported high metals levels (Pb, Cd, Hg, Cu) in mushrooms growing in heavily contaminated areas, such as those in the vicinity of highways with heavy traffic. The level of Cd from other report ranges from 0.3 to 3.0 µg/g [28] and 0.4 to 91.8 mg/kg [27] The Cd levels of Ethiopian mushrooms are in agreement with literature values. Cadmium is accumulated mainly in kidneys, spleen and liver and its level in blood serum increases considerably following mushroom consumption [2] Thus, the consumption of contaminated mushrooms such as *A.campestris* may pose a health risk for consumers, especially during the rainy season when intake is high (Table 3).

Lead was detected in all of the samples ranging from 1.52 mg/kg (*L.edodes*) to as high as 18.0 mg/kg (*A.bisporus*#1). The Pb concentrations in other studies were between 0.1 and 40 mg/kg [40,51]. Higher concentration of Pb in *Agaricus* species is also found by Chen et al., [27]. The uptake of heavy metal ions in mushrooms is higher than in plants. For this reason, the concentration variations of heavy metals could be attributed to mushroom species and their ecosystems. However, no mushroom species can be considered as an exact indicator of environmental pollution [2]. Certain countries have established

statutory limits for metals in edible mushrooms. In the Czech Republic, limits of 2.0 and 10.0 mg/kg dry matter have been established for Cd and Pb, respectively, in wild-growing mushrooms; whereas 1.0 and 10 mg/kg dry matter have been established for cultivated mushrooms [2]. In Poland, recommendations concerning the concentrations of Cd and Pb in dried mushrooms are slightly different. Tolerance limits set for Cd and Pb are 1.0 and 2.0 mg/kg dry weight, respectively [40]. The maximum level for certain contaminants in foodstuffs established by the Commission of the European Communities (Commission Regulation [EC] No 466/2001) is set at about 0.2 and 0.3 mg/kg wet weight for Cd and Pb, respectively, in cultivated fungi. Assuming that the dry matter content of mushrooms is 10% [2], these same limits for dry material will be ten times higher and approach 2.0 and 3.0 mg/kg dry weight for Cd and Pb, respectively. Thus, in Ethiopian mushrooms as shown in Table 3, the concentration of Cd for *A.campestris* (4.08 mg/kg) only exceeded the tolerance limits set by Czech, Poland and EU for dried mushrooms. In the contrary, the lead content in most of the fruiting bodies of mushroom analyzed was higher than the statutory limits set by EU and Poland except for *L.edodes* (1.57 mg/kg) which is safe.

Metal-to-Metal correlation

A linear regression correlations test was performed to investigate correlations between metal concentrations. The values of correlation coefficients between metal concentrations. There are good correlations between sodium and potassium (r=-0.41), sodium and phosphorus (r=-0.57), potassium and magnesium (r=0.41), potassium and phosphorus (r=0.71), potassium and zinc (r=-0.340, potassium and iron (r=0.42), potassium and manganese (r=0.39), potassium and copper (r=0.53), magnesium and phosphorus (r=0.51), magnesium and iron (r=0.34), magnesium and copper (r=0.54), phosphorus and copper (r=0.67), phosphorus and lead (r=0.34), zinc and cadmium (r=0.38), iron and manganese (r=0.87), copper and cadmium (r=0.33) and copper and lead (r=0.45). From these good correlations, except the negative correlations between sodium and potassium, sodium and phosphorus and potassium and zinc all other the correlations are positive. The other correlations between metals were not significant. Similar correlations between some of metals of mushroom were also reported by Chen et al. and Mendil et al., [27,39].

Daily mineral intakes and toxicity

According to the EU scientific committee for food adult weight parameter, 60 kg of body weight was used for intake calculations as the weight of an average consumer. In addition, for intake calculations, usually a 300 g portion of fresh mushrooms per meal is assumed, which contains 30 g of dry matter [2].

The significance to human health of the element concentrations found in fruiting bodies of edible fungi may also be assessed by comparing estimated exposures with exposures from a normal diet and with the internationally agreed exposure guidelines, where these exist [40]. These guidelines are the Provisional Tolerable Weekly Intakes (PTWIs) or Provisional Maximum Tolerable Daily Intakes (PMTDIs) set by the Joint Expert Committee on Food Additives (JECFA) FAO/WHO [52] or by EU Scientific Committee [53]. Acceptable weekly intakes of Cd and Pb for adults are 0.42–0.49 and 1.5–1.75 mg, respectively. These values correspond to 0.06 and 0.21 mg of Cd and Pb, respectively, on a daily basis.

Thus, in relation to the above FAO/WHO or EU guidelines, the PTWI for Cd assuming 60 kg body weight is 0.84 mg/week for *A.campestris* which is almost 2 fold of the weekly tolerance limit. Similarly, the PTWI for Pb for the analysed mushrooms: *P.ostreatus* (1.12 mg/week), *L.edodes* (0.35 mg/week), *L.sulphureus* (0.7 mg/week), *A.campestris* (1.82 mg/week), *T.clypeatus* (2.1 mg/week), *T.microcarpus* #1 (0.84 mg/week), *T.aurantiacus* (2.03 mg/week), *T.microcarpus*#2 (2.59 mg/week), *A.bisporus* #1 (3.78 mg/week) and *A.bisporus* #2 (2.45 mg/week) indicating most of the mushrooms samples exceeded the weekly tolerance limit set for Pb with more proportion in wild than cultivated mushrooms.

Anti-nutritional factors

Table 4 presents the antinutrients (phytate and tannin) distribution of the most commonly consumed cultivated and wild edible mushrooms collected from Ethiopia (Table 4).

Phytates: The phytate content varied from 31.3 mg/100 g for *A.bisporus*#1 to 242.8 mg/100 g for *A.bisporus*#2 in dry basis. From other reports the phytate concentration of cap, stalk and tuber of mushroom analyzed separately were in the ranges of 282 to 958 mg/100 g [24] and 338 to 1815 mg/100 g [23,25]. Aletor reported that phytic acid in tropical species ranged from 100 to 360 mg/100 g where samples were analyzed as a whole (without separating the cap and pileus). This report was in line with our report as the analysis of Ethiopian mushrooms was also carried as a whole. According to Akindahunsi et al. [54] the phytate content of mushrooms was low when compared to green leafy vegetable whose phytate content was exceptionally high. Phytic acid forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake because the first step in mineral absorption requires that the mineral remain in the ionic state [7] thus inducing mineral deficiencies. Since the level of phytic acid is low in the analyzed samples, mushrooms could be another possible option to combat mineral deficiencies in Ethiopia.

Sandberg et al. [55] suggested that food processing such as cooking, fermentation, autoclaving and milling can reduce or eliminate the level of phytic acid by altering the inositol hexaphosphate to other degradation forms, e.g. penta-, tetra-, tri-, di- and monophosphate. However, forms such as IP1, IP2 and IP3 have less capacity to bind metal ions, or the complex formed is more soluble, which may reduce the negative effect on mineral absorption [56,57]. Similar reduction in phytic acid was observed in one of our canned mushroom (*A.bisporus#2*) having the least phytic acid content of 31.3 mg/100 g. This reduction might be due to the leaching out of phytate to the water which was used to soak whole or sliced mushroom samples. A further investigation is needed to exactly evaluate the effect of heat, preservative (salt and

No.	Mushroom Type	Phytate	Tannin
1	*P.ostreatus*	155.8 ± 12.1	4.81 ± 1.35
2	*L.edodes*	104.4 ± 2.02	10.6 ± 0.46
3	*A.bisporus#1*	242.8 ± 54.9	31.7 ± 1.39
4	*A. bisporus#2*	31.3 ± 6.03	10.0 ± 1.26
5	*A.campestris*	235.3 ± 25.4	22.5 ± 0.99
6	*L.sulphureus*	147.6 ± 3.32	8.72 ± 0.83
7	*T.clypeatus*	106.5 ± 12.5	16.9 ± 0.97
8	*T.microcarpus#1*	119.8 ± 8.88	16.7 ± 1.12
9	*T.aurantiacus*	NA	NA
10	*T. microcarpus#2*	186.8 ± 28.9	26.1 ± 3.25
11	*T.letestui*	98.2 ±1.44	20.1± 0.69
12	*T.sp*	70.1 ± 1.33	32.5 ± 0.55

*NA= not analyzed

Table 4: Antinutrients content (mg/100 g) of cultivated and wild edible mushrooms collected from Ethiopia in dry basis.

citric acid) and water used in canning of mushroom to the reduction of antinutrients such as phytate and tannin.

Condensed tannins: Tannins are known to retard growth through reduced digestion and/or absorption [58]. Condensed tannin content expressed as (+) - catechin equivalent was varied from 4.81 mg/100 g (0.05%) for *P.ostreatus* to 32.5 mg/100 g (0.32%) for T.sp. From other reports the concentration of condensed tannin of the cap, stalk and tuber of mushroom where analyzed separately were in the ranges of 0.1% (10 mg/100 g) to 1.1% (110 mg/100 g) [24] and 0.21% (21 mg/100 g) to 0.31% (31 mg/100 g) [25]. Akindahunsi et al.[25] and Aletor [23] reported that tannin concentrations (%TA) in mushrooms were low. These levels might not affect the nutritional potentials of the mushroom parts since they were all less than 10% of the total dry weight of the samples [59].

Molar ratios and bioavailability of minerals: The molar ratio of phytate/mineral of all mushroom samples analyzed are summarized and shown in Table 5. The molar ratios of [phy]: [Zn] of all mushrooms samples were <10 indicating a good zinc bioavailability. Similarly the molar ratio of [phy]: [Ca] of <0.24 indicating good calcium bioavailability also. Moreover calcium promotes zinc bioavailability in all the samples since molar ratio of [Phytate]*[Ca]: [Zn] were <0.5 mol/kg. For iron content, all mushroom samples had good bioavailability except *A.bisporus*#1 and *P.ostreatus* with [Phytate]: [Fe] molar ratio >1 (Table 5).

Phy: Zn

The importance of a foodstuff as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH [60]. The formation of the chelates depends on relative levels of both zinc and phytic acid [61]. Hence, the phytate: Zn molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone[62]. Oberleas et al., showed that foods with a molar ratio of Phy: Zn less than 10 showed adequate availability of Zn and problems were encountered when the value was greater than 15. In Table 5, the Phy: Zn molar ratios are shown for the cultivated and wild edible mushrooms analysed. All the mushroom samples analyzed had low Phy: Zn values which is less than the critical value 15. This means that Zn obtained from these mushrooms would be bioavailable for the human body.

Phy: Fe

Phytate is known to be the main inhibitor of iron absorption in plant-based diets. To cancel out the adverse effect of phytates on iron absorption, phytate contents have to be reduced to concentrations of less than 0.1 g/100 g. In cereal-based dishes, it has been shown that the phytate/iron molar ratio has to be lower than 1, and preferably 0.4, to obtain a significant increase in absorption [63]. With this regard, most of our mushroom samples had phy: Fe molar ratios lower than 1 with the exception of *A.bisporus*#1 and *P.ostreatus* (Table 5). This indicates iron from these mushroom would be bioavailable to the human body.

Phy: Ca

Phytic acids markedly decrease Ca bioavailability and the Phy: Ca molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Phy: Ca is reported to be 0.24 [16]. The molar ratios of Phy: Ca obtained for both cultivated and wild edible mushrooms of Ethiopia (Table 5) were all less than the critical value indicating that absorption of calcium won't be adversely affected by phytate in these mushroom species.

Phy*Ca: Zn

A kinetic synergism exists between [Ca] and [Zn] ion resulting in a Ca: Zn: phytate complex, which is less soluble than phytate complex formed by either ion alone [60]. Table 5 presented the values for the molar ratios of [Ca] [Phy]/ [Zn] i.e. (Ca x Phy:Zn) [21]. Ellis et al. and [20] Davies et al. indicated that the ratio of Ca x Phy: Zn is a better predictor of Zn availability and said that, if the value were greater than 0.50 mol/kg, there would be interferences with the availability of Zn. In our results, the calculated Ca x Phy: Zn values were all lower than 0.50 mol/kg for the edible mushrooms analyzed. This means, Zn availability would not be affected by the presence of calcium. This bioavailability of zinc might be related to the level of phytate present in the samples were not high enough to adversely affect it. Similar bioavailability of Zn in all mushroom samples was revealed by [24]. Ola et al. and reported that the calculated [Ca] [phytate]/[Zn] molar ratio is a better index for predicting Zn bioavailability.

Phytate and non phytate P

Table 6 reveals the proportion of phytate and non phytate phosphorus in the mushroom samples in mg/100 g in dry basis. The proportion of phytate and non phytate phosphorus in the mushroom samples in mg/100 g. Phytate phosphorus ranged from 8.86 mg/100 g (12.4%) for *A.bisporus*#2 to 67.9 mg/100 g (24.2%) for *A.bisporus*#1, while non phytate phosphorus ranged from 61.9 mg/100 g (87.6%) for *A.bisporus*#2 to 235.5 mg/100 g (88.8%) for *T.clypeatus*. Generally diets are regarded as being adequate in bioavailable phosphate. However,

No.	Mushroom Type	Phytate: Zn	Phytate : Fe	Phytate : Ca	Phytate*Ca: Zn
1	*P.ostreatus*	2.47 ± 0.15	1.82 ± 0.12	0.08 ± 0.01	0.071 ± 0.00
2	*L.edodes*	1.27 ± 0.02	0.90 ± 0.03	0.03 ± 0.00	0.066 ± 0.00
3	*A.bisporus#1*	2.93 ± 0.37	3.26 ± 0.43	0.07 ± 0.00	0.169 ± 0.04
4	*A. bisporus#2*	1.16 ± 0.09	0.81 ± 0.08	0.01 ± 0.00	0.049 ± 0.00
5	*A.campestris*	2.65 ± 0.30	0.12 ± 0.01	0.16 ± 0.2	0.060 ± 0.00
6	*L.sulphureus*	3.76 ± 0.19	0.75 ± 0.07	0.11 ± 0.01	0.075 ± 0.00
7	*T.clypeatus*	1.36 ± 0.15	0.22 ± 0.02	0.07 ± 0.01	0.032 ± 0.00
8	*T.microcarpus#1*	1.92 ± 0.15	0.02 ± 0.03	0.09 ± 0.02	0.039 ± 0.00
9	*T.aurantiacus*	NA*	NA	NA	NA
10	*T. microcarpus #2*	2.19 ± 0.39	0.05 ± 0.01	0.13 ± 0.03	0.048 ± 0.00
11	*T.letestui*	1.29 ± 0.01	0.07 ± 0.00	0.11 ± 0.01	0.018 ± 0.00
12	*T. sp*	0.93 ± 0.02	0.01 ± 0.00	0.14 ± 0.00	0.053 ± 0.00

*NA=not analyzed

Table 5: Molar ratio phytate: mineral of wild and cultivated mushrooms analysed.

No.	Mushroom Type	Total P	Phytate P [a]		Non phytate P [b]	
		mg/100 g	mg/100 g	%	mg/100 g	%
1	*P. ostreatus*	250.0 ± 3.33	43.6 ± 3.38	17.4	206 ± 3.10	82.6
2	*L. edodes*	116.5 ± 1.75	29.2 ± 0.57	25.1	87.2 ± 1.55	74.9
3	*A. bisporus #1*	282.8 ± 3.33	67.9 ± 8.88	24.2	213.8 ± 11.7	75.8
4	*A. bisporus #2*	70.7 ± 3.45	8.86 ± 0.97	12.4	61.9 ± 2.80	87.6
5	*A. campestris*	214.0 ± 11.3	65.9 ± 7.10	31.0	148.1 ± 13.8	69.0
6	*L. sulphureus*	134.6 ± 3.56	41.3 ± 0.93	30.8	93.3 ± 4.27	69.2
7	*T. clypeatus*	265.3 ± 2.92	29.8 ± 3.50	11.2	235.5 ± 3.05	88.8
8	*T. microcarpus #1*	215.5 ± 20.9	33.5 ± 2.50	16.1	182.9 ± 23.4	83.9
9	*T. aurantiacus*	135.9 ± 0.07	NA		NA	
10	*T. microcarpus #2*	198.4 ± 10.9	52.3 ± 8.09	26.6	146.0 ± 14.7	73.4
11	*T. letestui*	208.5 ± 6.08	27.5 ± 0.69	13.2	180.9 ± 5.71	86.8
12	*T. sp*	235.8 ± 4.52	19.6 ± 0.37	8.3	216.2 ± 4.16	91.7

*NA=not analyzed

a=phytate phosphorous calculated by assuming phytate contains 28.18 % phosphorus (phytate *0.28)

b=non phytate phosphorous = total phosphorous - phytate phosphorous

Table 6: Concentration of phytate P and non phytate P cultivated and wild edible mushrooms collected from Ethiopia in dry basis.

the high proportion of phosphate as phytate has consequences for bioavailability of minerals and trace elements [64]. In the edible mushrooms evaluated the proportion of phosphate as phytate is low, hence phosphorus from these mushrooms could be bioavailable for the human body (Table 6).

Conclusions

This study has shown that the mushrooms collected from Ethiopia have good mineral concentrations with lower anti-nutritional factors that make them bioavailable to the human body. However, consumption of some contaminated mushroom should be avoided as they may pose a health risk for consumers. Thus, the outcomes obtained from the study clearly shows there is a great health and nutritional benefits from the consumption of edible mushrooms.

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