Original Research Article

Bio accessibility of trace elements in different oyster mushroom varieties grown in Kenya

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ABSTRACT

Trace elements, especially chromium (Cr), vanadium (V) and selenium (Se) have potential beneficial effects on glucose metabolism in people with type 2 diabetes. Food products incorporating mushrooms are not only a good source of such nutrients but are thought to have readily bioavailable nutrients. Nutritional efficacy of food products may be ensured by accessing bio accessibility of nutrients, which provides valuable information on matrix and appropriate dosage. The study determined bio accessibility of Cr, V and Se in four varieties of oyster mushrooms *Pleurotus Osteatus* (PO), *Pleurotus sajor caju* (PS), *Pleurotus Pulmonaries* (PP) and *Pleurotus Cintropiletus* (PC)) grown in Kenya. Bio accessibility was estimated using invitro simulated gastrointestinal procedure, while nutrient levels were determined using an atomic absorption procedure. Bio accessible levels of chromium ranged from 26.56% in PS to 78.50% in PC; selenium from 92.52% in PC to ND in PS and PP; and vanadium from 92.46% in PC to 69.95% in PP. Vanadium was had the highest bio accessibility in the four oyster mushrooms, more than other elements, while chromium had high bio accessible in PC variety

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Keywords: Bio accessibility, Oyster mushrooms varieties, Vanadium, Chromium, Selenium

Introduction

Bioaccessibilty can be defined as quantity or fraction which is released from the food matrix in the gastrointestinal tract and becomes available for absorption (Heaney, 2001; Onyambu *et al.*, 2021; Nambafu *et al.*, 2021). Bioavailability on the other hand is the ingested fraction available at the site of action for utilization in normal physiological functions; usually determined through *in vivo* assays (Guerra *et al.*, 2012; Lusi *et al.*, 2013). It is the result of three main steps: digestibility and solubility of the element in the gastrointestinal tract; absorption of the element by the intestinal cells and transport into the circulation; and incorporation from the circulation to the functional entity or target (Wienk *et al.*, 1999; Etcheverry *et al.*, 2012). Bioccessibility is usually evaluated by *in vitro* digestion procedures generally simulating gastric and small intestinal digestion, sometimes followed by Caco-2-cell uptake (Courraund *et al*., 2013). *In vitro* methods are developed to simulate the physiological conditions (temperature, agitation, pH, enzyme, and chemical composition) and the sequence of events that occur during digestion in the human gastrointestinal tract (Fernandez Garcia *et al*., 2009). The bioaccessible level of trace elements after consumption is not necessarily related to the levels in foods since food processing and cooking affect intestinal transit time of nutrients as well as their enteric formation of mixed micelles (Nambafu *et al*., 2021, Nawiri *et al*., 2013). In this study a static method was used the method involved invitro enzymolysis procedure simulating human gastro intestinal which involves two steps (gastric and intestinal).

Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. Bano (1976) suggested that food value of mushrooms lies between meat and vegetables. The fruiting bodies of mushrooms are characterized by a high level of well assimilated mineral elements. Major mineral constituents in mushrooms are K, P, Na, Ca, Mg

and elements like Cu, Zn, Fe, Mo, Cd form minor constituents (Bano and Rajarathanum, 1982; Bano *et al*., 1981; Chang, 1982). The present use of mushrooms is totally different from the traditional use because lot of research has been done on the chemical composition of mushrooms, which has revealed that mushrooms can be used as a diet to combat diseases. The early history regarding the use of mushrooms in different countries has been reviewed by a number of workers (Buller, 1915; Rolfe and Rolfe, 1925; Singer, 1961; Atkinson, 1961; Bano *et al*., 1964; Jandaik and Kapoor, 1975; Bano and Rajarathnam, 1982; Abou *et al*., 1987; Houghton, 1995). The mineral proportions vary according to the species, age and the diameter of the fruiting body. It also depends upon the type of the substratum (Kalac and Svoboda, 2000). The mineral content of wild edible mushrooms has been found higher than cultivated ones (Aletor, 1995; Mattilla *et al*., 2001; Rudawska and Leski, 2005). The minerals have been found to boost the immune system, have anti-cancerous properties, and act as anti-hypercholesterolaemic and hepatoprotective properties. Some mushrooms such as *Pleurotus* species are excellent food for the people suffering from hypertension and cardiovascular diseases due to high potassium and sodium content (Ganesan and Xu, 2018). Chromium is generally recognized to play an important role in glucose and lipid metabolism. One effect of chromium is that it is very helpful in preventing and reversing type 2 diabetes because plasma glucose is more effectively regulated in the presence of chromium (Anderson *et al*., 1997).

Vanadium compounds have been demonstrated to mimic the action of insulin in isolated cell systems, animal models and diabetic patients. This has brought the use of V compounds as potential sources of diabetes therapy into focus (Shechter, 1990 and Shamberger, 1996). Selenium have been shown to reduce the risk of developing dysglycemia- a broad term that

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refers to any abnormalities in blood glucose levels leading to disease (Akbaraly *et al*., 2010). It is also thought to work by exerting insulin-like actions in vitro (Becker, *et al*., 1996).

In Kenya hypertension and diabetes are becoming the major causes of morbidity and mortality with their prevalence surpassing that of HIV/AIDs (WHO, 2012). Various mushrooms species are used in the treatment and prevention of diabetes, due to their rich nutritional and medicinal properties such as the elements (K, Ca, Na, P, Fe, K, Cr, Se, and V) (Rajarathnam and Shashirekha, 2011). The management of diabetes through use of nutraceuticals requires that the substrate not only contain high levels nutrients but also bioavailable nutrients. Since the levels of the nutrients depend on growing medium, variety, maturity of tissue and climatic condition it is important to quantify the levels in various species of mushrooms. Mushrooms have a chitin structure that is likely to have effect on the micronutrients bioavailable. Bioavailability of nutrients depends on various factors such as food tissue and on host related factors and is studied through intervention (*in vitro* procedures), or algorithms and simulated gastrointestinal digestion (*in vivo* methods). Bioavailability is improved by formulating a food product containing high levels of nutrients such Cr, V and Se that are important in management of diabetes. This study was conducted to determine the bioaccessibility of trace elements (chromium, vanadium, and selenium) in the fresh mushrooms commonly consumed in Kenya.

2. Experimental

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2.1 Samples and sample preparation

Four oyster mushroom (*pleurotus ostreotus*) varieties were collected from nearby farms in Jomo Kenyatta University and identified by a taxonomist. The freshly harvested mushrooms were

cleaned, cut into pieces and dried in the shade for four days and then powdered before keeping in a labeled special glass container awaiting analysis

2.2 Reagents and Apparatus

All reagents were of analytical reagent grade. Double deionized water (Milli-Q Millipore 18.2 MX/cm) was used in dilution of reagent solutions. HNO_3 and H_2O_2 were of supra pure quality (Merck). Gastric juice, Pepsin (1% w/v), Pacreatin (3% w/v), Amylase (1% w/v), Bile salt, and selenium, chromium and vanadium as standards were purchased from Sigma Andrich. All plastics and glassware were cleaned, soaked in dilute HNO_3 (10%) and rinsed with distilled water prior to use.

2.3 Instrumentation

AAS spectrophotometer, Thermo Jarell Ash (model AAS S11) was used for analysis of trace elements. The most appropriate wavelength, hallow cathode lamp current gas mixture flow rate, slit width and other AAS instrument parameters for metals, minerals were selected as given in the instrument user's manual and background correction was used during determination of metals/minerals.

2.4 Procedures of analysis

2.4.1 Method validation

Calibration of AAS was done using the working standard prepared from commercially available metal/mineral standard solutions (1000µg/ml. The stock solutions were kept under refrigeration conditions to be used for analysis. The working solutions of different concentrations were

prepared daily by serial dilution of the standard. Various volumes of the stock solutions in each case (2, 4, 6, 8 ml) were further diluted to 100 ml to obtain the working solutions of concentrations 50 ppm, 100 ppm, 150 ppm and 200 ppm. After filtering absorbances were obtained and used to draw calibration curves. All analyses were done in triplicates.

2.4.2 Determination of trace elements

Each mushroom sample was air-dried at 105 °C overnight and crushed using a mortar and pestle into powder. Digestion of the mushroom samples was performed using a mixture of HNO_3:H_2O_2 (10:1, 12 ml g^{-1} of sample) and heated at 100 °C for between 10-15 min. After cooling, the solution was made to 50 ml with deionized water after filtration. The amounts of Cr, Se and V were determined using an atomic absorption spectrometer. All analyses were done in triplicates.

2.4.3 *In vitro* simulated gastrointestinal digestion

The *in vitro* enzymolysis procedure simulating human gastrointestinal digestion was carried out in triplicate. Procedural blanks were run to check the presence of Se, Cr and V in the reagents. Mushroom samples (0.5 g) in a flask were incubated with 5 ml of gastric juice (1% w/v pepsin in 0.15 M NaCl, adjusted to pH 2 with HCl (37% v/v) and, after 1 min of vigorous shaking for initial degassing, the flask was placed in a mixing water bath (GFL 1083, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at 37^0 C for 4 h (Crews *et al.*, 1996). The solution was then adjusted to pH 6.8 with $NaHCO_3$. After adding 5 ml of intestinal juice (3% w/v pancreatin, 1.5% w/v amylase, 1% w/v bile salts in 0.15 M NaCl), solution was vigorously shaken for 1 min, degassed and further incubated for 4 h at 37 ^{0}C under gentle shaking. The solution was then centrifuged at 8000 g and 4^0 C for 15 min, the supernatant collected, filtered through 0.45 µm membranes, and stored at -80 ^{0}C until analysis. The amount of solubilized chromium, selenium

and vanadium in the supernant was measured using atomic absorption spectrophotometer as a measure of their bioaccessibility.

3 Results and Discussion

3.1 Method validation

Calibration curves shows the response of analytical method to known quantities of analyte (Harris,2007).The calibration curves for chromium, selenium and vanadium were linear within the concentration range determined.The calibration line for Cr gave a correlation coefficient r^2 = 0.992 and regression equation y =0.058x+0.003 The calibration line for selenium gave a correlation coefficient r^2 = 0.992 and regression equation Y=0.023x+0.000.The calibration line for vanadium gave a correlation coefficient r^2 = 0.9975 and regression equation y=0.0143x (Table 1).

Tables 1:Validation parameters

Element	R^2	Regression Equation
Cr	0.992	Y=0.058X+0.003
Se	0.992	Y=0.023X+0.000
Va	0.9975	Y=0.014X

The calibration regression procedure was used for analysis and gave correlation coefficiencies ranging between r^2 = 0.992 and 0.9975, representing acceptable linearity for determination. However, when the correlation coefficient is zero does not mean that y and x are entirely

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Comment [H7]: In my opinion, there is a good discussion of the results.

However, there is an excess of information (related to bibliographic review) and not to the discussion of the results with respect to other research.

Finally, most of the bibliographic citations are old.

Comment [H8]: Personally I consider that this section (highlighted in yellow) is not necessary, it is understood that from the calibration curves (mentioned in materials and methods), the equation of the line was used to make the pertinent calculations.

unrelated, it only means they are they are not linearly related. The closer the y values to one the linear the value (Harris, 2007).

3.2 Levels of trace elements in oyster mushrooms

The mean trace element concentrations (µg/g) in dry weight in the mushroom samples were Chromium PSC (1.551), PP (1.798), PO (3.501), and PC (1.430). Vanadium concentrations in the four species were PS (2.338), PP (0.776), PO (1.165) and PC (1.110). Selenium levels were PSC (0.471), PP (0.606), PO (0.488) and PC (0.450) (Table 2). The precision of the results was evaluated based on the standard deviation of the results of triplicate samples (n=3) analyzed under the same conditions mean±SD. The mean concentration of chromium was highest in PO and lowest in PP. Selenium concentration was highest in PSC and lowest in PP. Vanadium concentration was highest in PC and lowest in PP. The values are comparable to those discussed by authors (Patil *et al*., 2010; Yang *et al*., 2001; Mshandete and cuff, 2008; Ahmed *et al*., 2009; Alam *et al*., 2008; Gosh and Chakrvarty, 1990; Gasecka *et al*., 2016; Kortei and Wiafe-kwangan, 2015; Bano *et al*., 1981; Jegadeesh *et al*., 2018, Ijeh *et al*., 2009).

Table 2: Mean levels of trace elements in Oyster mushrooms

Mushroom variety	**Concentration (µg/g)**		
	Chromium Mean±SD	Vanadium Mean±SD	Selenium Mean
PSC	1.551±1.661	0.471±1.481	2.338±0.055
PP	1.798±1.472	0.606±3.341	0.776±0.066
PO	3.501±0.857	0.488±0.946	1.165±0.01
PC	1.430±0.657	0.450±2.472	1.110±0.06

PO *Pleurotus Ostreatus*,PSC *Pleurotus Saju Caju*, PP *Pleurotus Pulmonarius*,PC *Pleurotus cintrinopileatus*

3.3

Table 3:Anova and F test

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I personally believe that table 3 could be eliminated, since it does not provide outstanding information.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
CHROMIUM	Between Groups	19.656	7	2.808	5.946	0.002
	Within Groups	7.557	16	0.472		
	Total	27.213	23			
VANADIUM	Between Groups	0.093	7	0.013	9.094	0.000
	Within Groups	0.023	16	0.001		
	Total	0.117	23			
SELENIUM	Between Groups	816.838	7	116.691	2.639	0.051
	Within Groups	707.556	16	44.222		
	Total	1524.394	23			

Table 4: Bioaccessible selenium

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Mushroom Variety	Level of Selenium Mean (Number)	
	Bio	Raw
PC	14.8466 (3)	1.1102(3)
PO	8.91368.9136(3)	1.1652(3)
PP	-1.3883(3)	0.7757(3)
PSC	-5.1000(3)	2.3377(3)

Table 5: Bioaccessible Vanadium

Mushroom Variety	Level of Vanadium Mean (Number)

	Bio	Raw
PC	0.4159(3)	0.4498(3)
PO	0.4138(3)	0.4880(3)
PP*	0.4237(3)	0.6057(3)
PSC	0.4011(3)	0.4710(3)

Table 6: Bioaccessible Chromium

Mushroom Variety	Level of Chromium Mean (Number)	
	Bio	Raw
PC	1.1221(3)	0.9506(3)
PO*	1.0025(3)	3.5010(3)
PP	0.6789(3)	0.7478(3)
PSC	0.4119	0.9559

*. The mean difference is significant at the 0.05 level. Based on *post hoc* Turkey HSD

3.4 Trace elements Bioaccesibility

Absorption of selenium occurs throughout gastrointestinal tract, with duodenum as a major site (Humaloja and Mykkänen, 1986). Selenium absorption is very efficient, normally in the range of 26.3% - 97% (Durcos *et al.*, 2005; Thompson *et al.*, 1978), with higher retention of organic

selenium compound than inorganic selenium compounds (Finely, 1999). Very little is known about mechanism of selenium absorption, it may involve both carrier mediated process for organic compound and diffusion controlled process for inorganic selenium compound (Arduser *et al*., 1985: Raghib *et al*., 1986).

The amounts of selenium bioaccessible in the four oyster mushrooms are as shown in Table 7 are PO-86.94%, PC-92.52% which is high and is comparable to literature value (Durcos *et al.*, 2005; Thompson *et al*., 1978) and thus is potentially bioavailable. The other varieties, PS and PP had undetected levels. The soluble extract obtained after *in vitro* simulated gastrointestinal digestion of the selenized mushroom contained about 106 g Se dry weight. This means that 75% of the Se taken up by the mushroom was solubilized in conditions simulating human gastrointestinal digestion and thus was potentially bioavailable (Hur *et al*., 2011). Se content of mushrooms is generally higher than that of most vegetables (Rayman *et al.*, 2008) but it is very variable. Apart from the bioavailable concentrations in soil, the amount of selenium found in wild edible mushrooms is dependent on the species and the stage of maturity; for cultivated species the substrates used for growth are important (Kalac, 2009).

Selenium occurs mostly as -2, +4 and +6 oxidation states and forms covalently bounded compounds with C-Se and Se-S bonds. These chemical forms of selenium determine not only the elements bioavailability but also its metabolic fate, distribution, nutritional importance (accessibility) for functional seleproteins accumulation and toxicity (Thomson, 1998; Suzuki *et al*., 2006). Selenium as selenomethionine in solution is actively transported (mechanism shared with methionine) with a yield of above 90%, and about 60% respectively (Thomson, 1998). Some dietary factors can influence the absorption rate of selenium such as presence of vitamin C which hampers selenite absorption. Dietary allowance for selenium is 0.87µg/kg body weight

(60.9 µg/person; 70kg body weight (Whanger, 2002). The recommended daily allowance is 55 µg Se/person for health adults. Adverse health effects may only occur at daily dosage of 900-1600µg/person, and selenosis occurs at 3200-5000µg/person (Combs, 2001; Whanger, 2002; Whanger, 2004)

Se that is not solubilized after gastrointestinal digestion might be present in form of indigestible Se-containing polysaccharides. For instance, it has been shown that part of the Se in Se-enriched mycelia of Pleorotus ostreatus is associated with chitin-containing structures in cell walls (Munoz *et al*., 2006). Formation of Se-containing polysaccharides might explain the low Se bioavailability found elsewhere for other species (Chansler *et al*., 1986; Mutanen, 1986). Limited bioavailability of Se in these species might be the result of a low bioaccessibility due to a larger incorporation of Se in mushroom polysaccharides compared to *Pleorotus ostreotus* in the conditions of the present study. From the nutritional point of view, selenium is one of the potential sources of nutrients that work as a cofactor in antioxidants. Bioavailability of selenium from PO, PF, PSC, and PE was found to range between 0.011 and 0.512mg/100 g (Ga secka *et al*., 2016; Tang *et al*., 2006). There is unclear evidence that suggests that selenium may reduce the incidence of cancer when taken in higher doses.

Vanadium has a similar character to that of phosphate and is present in several oxidation states at +2∼+5, many types of compounds have been prepared. Compounds with a +5oxidation state are most stable (Kustin and Macara, 1982). Usually, vanadium ions bind with the oxygen atom to form oxo compounds such as VO_3^- as vanadate and VO^{2+}as vanadyl forms. The vanadic form of $V^{3+}$is very unstable under air and oxidizes to vanadyl or the vanadate form (Chasteen, 1983; Kustin and Macara, 1982; Rehder, 1995). Humans usually take vanadium at 10-60mg through foods daily, and 50-200mg of vanadium is estimated to be found in the human body. In each

organ, vanadium is present at 0.01-1mg and contributes to a wide variety of physiological roles. In tissues, ~90% of vanadium is bound with proteins and 10% is present as low molecular ionic forms (Stern *et al*., 1993).

The bioaccessible vanadium in the four oyster mushrooms in this study was PS-85.16%, PP-69.95%, PO-84.80% and PC-92.46% (Table 7). Many studies confirm that of the total dietary vanadium ingested, less than 5% is actually absorbed by the gastrointestinal tract (GI) (Curran *et al*., 1990; Byrne, 1978; Nielsen, 1988). Other studies claim greater than 10% of ingested vanadium may have resulted in greater than normal absorption efficiencies (Nielsen, 1990). The majority of the 5% dietary vanadium absorbed is taken up by the upper GI tract (Patterson, 1986). This low absorption value should not imply, however that high levels of dietary vanadium are not without deleterious effect. High vanadium intake in animal models is known to both influence and be affected by the gastrointestinal metabolism of chloride, iodide, chromium, iron, copper, ascorbic acid, cysteine, methionine, riboflavin and some proteins.

Chromium (Cr) is an ubiquitous metal, occurring in water, soil and biological systems. The three most stable forms of chromium occurring in the environment are: 0, +3, and +6 valence state; metal and alloys, trivalent chromium, and hexavalent chromium, respectively (European Commission, 2003; Mertz and Cornatzer, 1971, Zha *et al*., 2007). Trivalent chromium is considered to be an essential element, both in animal feeding and human nutrition. This trace element is involved in the metabolism of carbohydrates, lipids, and proteins mainly by increasing the efficiency of insulin (Offenbacher, 1994). Chromium deficiency affects the maintenance of normal glucose tolerance and healthy lipid profiles. The suggestion that Cr intake is generally low has generated interest regarding the supposed beneficial effects of Cr supplementation on biological function and health of animals and humans (Ducros, 1992). In the USA in 2001, the

dietary guidelines for daily chromium uptake was lowered from 50-200 for adults to 35 and 25 µg for men and women, respectively (Food and Nutrition Board Institute of Medicine, 2000).

Table 7: Bioaccesibility of elements in four oyster mushrooms

Mushroom variety	**Bioaccessibility (%)**		
	Chromium	Vanadium	Selenium
PSC	26.56	85.16	ND
PP	37.59	69.95	ND
PO	28.63	84.80	6.94
PC	78.50	92.46	9.52

ND: *Not Detected*

The bioaccessible chromium levels in the four oyster mushrooms were PS-26.56%, PP-37.59%, PO-28.63% and PC-78.50% (Table 7). These values are comparable to those in literature in *in vitro* and in vivo studies in rats have shown that about 80% Cr in the blood is associated with transferring (Feng, 2003). Chromium is absorbed together with other metal ions in the gut through the unsaturated passive transport. The absorption process depends on the Cr content in the diet and on the chemical form of this element and other food components (Dowling *et al*., 1989). The efficiency of this process is very low with the average absorption ranging from 0.4-2.5% (European Commission, 2003). The absorption process depends on the Cr content in the diet and on the chemical form of this element and other food components. Studies conducted in rats showed increased absorption of Cr used in the form of nicotinate (1.3%) and picolinate (1.1%) in comparison to chromium chloride (0.9%) (Anderson, 1996). It was shown that absorption of Cr in humans in the form of chromium chloride is much lower (0.1-0.4%) than of chromium picolinate (2.8%) or chromium given as the yeast chromium (5-10%) (European Commission, 2003; Mertz and Cornatzer, 1971). Organic sources of Cr (picolinate, or

propionate-methionine salt) are much better absorbed than inorganic forms (oxides), and lead to the increase of these compounds' concentration in tissues (Ohh and Lee, 2005). The highest dose-accumulation correlation of Cr in the tissues is observed after administration of Cr nanoparticles (Wang *et al*., 2009; Zha *et al*., 2007). However, other factors present in the diet show a significant impact on the amounts of Cr absorbed from the gastrointestinal tract. Starch, simple sugars, ascorbic acid, oxalic acid, nicotinic acid, some amino acids, aspirin increase absorption of this element (Chen *et al*., 1973; Davis *et al*.,1995; Offenbacher,1994; Samanta, 2008) while high concentrations of phosphate, calcium, magnesium, titanium, zinc, vanadium and iron reduce the rate of this process (Chen *et al*., 1973; Hill, 1976).

After absorption from the intestine, chromium (III) is released into the bloodstream where it is bound by proteins involved in iron metabolism. *In vitro* and *in vivo* studies in rats have shown that about 80% Cr in the blood is associated with transferring of chromium III (Feng, 2003). In this complex, Cr is transported to the cells, and the efficiency of Cr transfer through the cell membrane depends on insulin concentration (Clodfelder and Vincent, 2005). Chromium is found in all animal tissues and is present at the concentrations of several to tens of μg/kg, rarely exceeding 100 μg/kg (National Research Council, 2005). The highest concentrations are found in the liver, kidneys and spleen, while slightly lower levels are observed in heart, muscle, pancreas, lungs, bones and brain (Feng, 1988; Feng, 2007).

More than 80% of Cr is removed from the body in the form of urine, while the remaining part of this element is excreted via faeces and sweat (Ducros, 1992). In humans, consumption of large amounts of sugar, exhaustive physical exercise, pregnancy and lactation leads to increased Cr excretion in the urine (Anderson, 1989).

4 CONCLUSIONS

The study indicates that the oyster mushroom contains fairly high levels of the elements but their bio accessibility differs in all the varieties. The extent of bio accessibility of the elements was affected by the variety although selenium remained least bio accessible in all varieties. Vanadium was better bio accessible in the four oyster mushrooms, while chromium was better bio accessible in the PC variety. Although bio accessibility of chromium was not as high as for vanadium the levels bio accessible are still beneficial in controlling the incidence and management of type 2 diabetes. A regular consumption of these mushrooms may therefore be promoted as a healthy diet.

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Comment [H11]: Verify that all references are written according to the journal's standards.

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