

Original Research Article

Bioaccessibility of trace elements in different oyster mushroom varieties grown in Kenya

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 **bioaccessibility** of nutrients, which provides valuable information on matrix and appropriate dosage. The study determined **bioaccessibility** of Cr, V and Se in four varieties of oyster mushrooms: *Pleurotus ostreatus* (PO), *Pleurotus sajor-caju* (PS), *Pleurotus pulmonarius* (PP) and *Pleurotus citrinopileatus* (PC) grown in Kenya. **Bioaccessibility** was estimated using in vitro simulated gastrointestinal procedure, while nutrient levels were determined using atomic absorption procedure. **Bioaccessible** 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 the most bio accessible than the other elements in the four oyster mushrooms, while chromium was the most bio accessible had high bio accessible in PC variety.**

Keywords: **Bioaccessibility**, Oyster mushrooms varieties, Vanadium, Chromium, Selenium

Introduction

Bioaccessibility 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; Onyambuet *al.*, 2021; Nambafuet *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 *etal.*, 2012; Lusiet *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 (Wienket *al.*, 1999; Etcheverry *et al.*, 2012). **Bioaccessibility** is usually evaluated by *in vitro* digestion procedures generally simulating gastric and small intestinal digestion, sometimes followed by Caco-2-cell uptake (Courraundet *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 (Nambafuet *al.*, 2021, Nawiriet *al.*, 2013). **In this study a static method was used which involved *in vitro* enzymolysis procedure simulating human gastro intestinal involving gastrointestinal 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 (Samsudin and Abdullah, 2019; Kalac, 2013; Liet *al.*, 2021). The present use of mushrooms is totally different from the traditional use because a 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 (Samsudin and Abdullah, 2019; Murugesan, 2017; Chang and Wassers, 2017; Hoet *al.*, 2020; Kalac, 2013; Liet *al.*, 2021). 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 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, Cr, Se, and V)(Rajarithnam 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. Material and Methods

2.1 Experimental

2.1.1 Samples and sample preparation

Four oyster mushroom (*pleurotusostreotus*) 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.

2.1.2 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. HNO₃ and H₂O₂ 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 HNO₃ (10%) and rinsed with distilled water prior to use.

2.1.3 Instrumentation

AAS spectrophotometer, Thermo Jarell Ash (model AAS S11) was used for analysis of trace elements. The most appropriate wavelength, hollow 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.

2.2 Procedures of analysis

2.2.1 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.

2.2.2 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 HNO₃:H₂O₂ (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.

2.2.3 *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 37⁰ C for 4 h (Crews *et al.*, 1996). The solution was then adjusted to pH 6.8 with NaHCO₃. 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 37⁰ C under gentle shaking. The solution was then centrifuged at 8000 g and 4⁰ C for 15 min, the supernatant collected, filtered

through 0.45 µm membranes, and stored at -80°C until analysis. The amount of solubilized chromium, selenium, and vanadium in the supernatant was measured using atomic absorption spectrophotometer as a measure of their bioaccessibility and data analyzed using SPSS.

3 Results and Discussion

3.1 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 1). 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; Ahmed *et al.*, 2009; Alamet *et al.*, 2008; Gosh and Chakraborty, 1990; Gasecka *et al.*, 2016; Kortei and Wiafe-kwangan, 2015; Bano *et al.*, 1981; Jegadeesh *et al.*, 2018; Ijeh *et al.*, 2009).

Table 1: 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-sajur Caju*, PP *Pleurotus pulmonarius*,PC*Pleurotus cintrinopileatus*

3.32 Stastical analysis

The data was analysed using SPSS program. Data was analysed using analysis of variance (ANOVA). ANOVA is used to separate and estimate the different causes of variation, Anova can also be used in situations where there is more than one source of variation (Harris,2007). ANOVA tests whether the difference between the sample means is too great to be explained by the random error. If the null hypothesis is correct, these two estimates of variance should not differ significantly (Miller and Miller,1988). If it is incorrect, the between sample estimate of variance will be greater than the within sample estimate because of between sample variation (Miller and Miller,1988). In this study the between sample estimate of variance is greater than within sample that is Chromium between group (BG) variation was 2.808 while the within group variation (WG) was 0.472; Vanadium BG(0.013) and WG(0.001) and selenium BG(116.691) and WG(44.222) to be explained by random error hence a one-tailed F-test was calculated to test whether it is significantly greater. From the table of critical values of F for a one-tailed test ($p=0.05$) the critical value of F is 8.845. The calculated value of F(5.946 for chromium) and 2.639 is smaller than this the null hypothesis is accepted the sample means do not differ significantly while that for Vanadium is 9.094 is greater than the critical value. A significant result in one way anova can arise for several reasons: one mean may differ from others, all the means may differ from each other, the means may fall into two distinct groups. A simple way of deciding the reason for a significant result is to arrange the means in increasing order and compare the difference between adjacent values with a quantity called least significant difference (Miller and Miller,1988). The least significant difference method is not entirely rigorous, it can be

shown that it leads to rather too many significant difference. However it is a simple follow up test when ANOVA has indicated that there is a significant difference between means (Harris,2007). In this study the lowest mean was 0.450 and the highest was 3.501. The least significant difference test is used in the context of ANOVA when the F-ratio suggests rejection of the null hypothesis. Turkey, Students-Newman-Keuls (SNK) and Bonferroni Post Hoc test are used for reporting the range tests and pairwise multiple comparison to determine means that differ in ANOVA. Range tests identify homogeneous subsets of means that are not different from each other. Pairwise multiple comparisons test the difference in each pair of means and yield a matrix where asterics indicates significantly different group means at alpha level of 0.05. In this study Bonferroni post Hoc test was used (Table 2). In this study only PO variety in chromium showed a significant difference (Table 2).

Table 2: Bioaccessible levels of Chromium, Vanadium and Selenium

Mushroom Variety	Level of Chromium Mean (Number)		Level of Vanadium Mean (Number)		Level of Selenium Mean (Number)	
	Bio	Raw	Bio	Raw	Bio	Raw
PC	1.1221(3)	0.9506(3)	0.4159(3)	0.4498(3)	14.8466(3)	1.1102(3)
PO*	1.0025(3)*	3.510(3)*	0.4138(3)	0.4880(3)	8.91368(3)	1.1652(3)
PP	0.6789(3)	0.7478(3)	0.4237(3)	0.6057(3)	-1.3883(3)	0.7757(3)
PSC	0.4119(3)	0.9559(3)	0.4011(3)	0.4710(3)	-5.1000(3)	2.3377(3)

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

3.3 Trace elements **Bioaccessibility**

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% (Durcoset *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 carriers mediated process for organic compound and diffusion-controlled process for inorganic selenium compound (Arduseret *al.*, 1985).

The amounts of selenium bioaccessible in the four oyster mushrooms are as shown in **Table 3** are PO-86.94%, PC-92.52% which is high and is comparable to literature value (Durcoset *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 106gSe 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 (Huret *al.*, 2011). Se content of mushrooms is generally higher than that of most vegetables (Raymanet *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).

Dietary allowance for selenium is 0.87µg/kg body weight (60.9µg/person; 70kg body weight (Whanger, 2002). The recommended daily allowance is 55µg Se/person for health

adults. Adverse health effects may only occur at daily dosage of 900-1600 µg/person, and selenosis occurs at 3200-5000 µg/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 *Pleurotostreatus* 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 *Pleurotostreatus* 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 0.512 mg/100 g (Gasecka *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 +5 oxidation 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 10-60 mg through foods daily, and 50-200 mg of vanadium is estimated to be found in the human body. In each organ, vanadium is present at 0.01-1 mg 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 3). Many studies confirm that of the total dietary vanadium ingested, less than 5% is 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, Zhaet *et al.*, 2007). Trivalent chromium is 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 were 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 3: Bioaccessibility 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 3). These values are comparable to those in literature *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; Zhaet *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 transferrin 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 $\mu\text{g}/\text{kg}$, rarely exceeding 100 $\mu\text{g}/\text{kg}$ (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.

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