Original Research Article

Bioaccesibility 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 bioaccessible 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 from26.56% in PS to 78.50% in PC; selenium from 92.52% in PC to ND inPS and PP; and vanadium from92.46% 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 in the PC variety.

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

Introduction

"Bioaccesibility 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). "Bioaccesibility is usually evaluated by *invitro* 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 which involved *invitro* enzymolysis procedure simulating human gastro intestinal involving 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; Li *et 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; Ho *et al.*, 2020; Kalac, 2013; Li *et 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) (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. Material and Methods

2. 1Experimental

2.1.1Samples and sample preparation

Four oyster mushroom that is *Pleurotus ostreatus* (PO), *Pleurotus sajor-caju* (PS), *Pleurotus pulmonarius* (PP) and *Pleurotus citrinopileatus* (PC) 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 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, 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.

2.1.3 Instrumentation

Atomic Absorption Spectrophotometer (AAS), Thermo Jarell Ash (model AAS S11) was used for analysis of trace elements. The most appropriate wavelength, 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 absorbencies were obtained and used to draw calibration curves. All analyses were done in triplicates.

2.2.2Determination 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 spectrophotometer. 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 andV 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 at 37^0 C for 4 h (Crews *et al*., 1996). The solution wasthen 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 and data analyzed using SPSS.

2.2.4 Stastical analysis

The data was analysed using SPSS program.ANOVA was used to determine wether there is significant difference in the bioaccessibility of the trace elements. 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, and 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 comparisons 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 1).The PO variety in chromium showed a significant difference(Table 1).

Table 1: Bioaccessible levels of Chromium, Vanadium and Selenium

Mushroom Variety	Level of Chromium Mean (Number) ug/g		Level of Vanadium Mean (Number) ug/g		Level of Selenium Mean (Number) ug/g	
	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 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 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;Ahmed *et al.*, 2009;Alam *et al.*, 2008;Gosh and Chakrvarty,1990;Gaseck*aet 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-sajur Caju*, PP *Pleurotus pulmonarius*, PC *Pleurotus cintrinopileatus*

3.2Trace 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).

The amounts of selenium bioaccessible in the four oyster mushrooms are as shown in Table3 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 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 (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.

"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. Bioavailability of selenium from PO, PF, PSC, and PEwas found to range between 0.011 and0.512mg/100 g (Gasecka *et al*., 2016; Tang *et al*., 2006).

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% (Table3).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).

Table3: 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	86.94
PC	78.50	92.46	92.52

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 *invitro* 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" (Anderson, 1996). "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; 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).

"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 accessiblein the four oyster mushrooms, while chromium was better bio accessible in the PC variety.

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