Original Research Article

Proteins and mineral content of cultivated oyster mushrooms grown in Kenya

Abstract
Mushrooms are a good source of proteins, vitamins and minerals, and are low in fat and sugars. The various varieties have shown to contain 25 -50% protein and 8 – 12% minerals, with various photochemical compounds. The objective of the study was to assess the proximate composition, mainly crude proteins and trace elements in four varieties (*Pleorotus ostreatus* (PO), *Pleorotus sajor caju* (PS), *Pleorotus pulmonaries* (PP), *Pleorotus cintropiletus* (PC)) of dried oyster mushrooms. The crude protein compositions of the mushrooms ranged from 35.05% in (PC) to 36.86% in (PO). Mineral composition in the four mushrooms was potassium 54.85 – 73.03mg/kg, sodium 23.51 – 26.89mg/kg, manganese 0.611 - 0.69mg/kg, cobalt 1.13 - 1.19mg/kg, lead 0.95 - 1.24mg/kg , cadmium 0.17 - 0.18mg/kg , zinc 0.48 - 0.66 mg/kg, nickel 1.85 - 2.19mg/kg, chromium 1.33 - 1.64mg/kg , copper 0.17 - 0.35mg/kg, iron 1.17 - 1.91mg/kg, calcium 0.82 - 3.59mg/kg and magnesium 1.55 - 2.28mg/kg. The oyster mushrooms varieties grown in Kenya are rich in proteins and minerals.

Key word: Oyster mushroom varieties, protein content, trace elements content

1. Introduction

Fungi and especially mushrooms are rich sources of nutrients important to health. In addition to being tasty, edible mushrooms are nutritious and contain protein, carbohydrates, lipids, vitamins and minerals (Varo, 1980). Trace elements such as potassium, sodium, copper, iron, zinc and chromium, and vitamins such as riboflavin, niacin, foliate and vitamins C and D are important for regulation

and maintenance of the body mechanism for good health. Mushrooms are also high in fiber content. Since mushroom can convert agricultural wastes into nutritional food they can be used as a weapon against starvation in developing countries (Lelley, 1987). The cultivated mushrooms contain 30-50% protein on dry weight basis, which can play a constructive role in solving one of the main problems in the twentieth century, the need to feed an increasing population. Their protein is of good quality and contains all dietary essential amino acids. Oyster mushrooms (*pleorotus* species), the third largest commercially produced mushroom in the world (Van, 2009) have several nutritional and medicinal properties (Garcha *et al*., 1993). They have low sodium but more potassium and iron than most foods.

Mushroom are fungi and can be classified into three basic ecological groups, mycorrhizal, parasitic and saprophytic. Pleurotus species, commonly known as oyster mushrooms, are edible fungi cultivated worldwide especially in south east Asia, India, Europe and Africa(Mandeel *et al*.,2005). China produces 64 % of all edible mushrooms in the world and 85% of all oyster mushrooms all over the world (Pleurotus spp.) is also produced in China. China is the main producer of cultivated, edible mushrooms. Lentinus edodes is now the world's leading cultivated edible mushroom with about 22% of the world's supply. Lentinula and four other genera (Pleurotus, Auricularia, Agaricus, and Flammulina) account for 85% of the world's total supply of cultivated edible mushrooms (Chang, 1980; Royse et al 2017). Oyster mushrooms is the third largest (Obodai *et al*., 2003) commercially produced mushroom in the world; however, Sánchez, 2010 reported that *Pleurotus ostreatus* is the second largest next to *Agaricus bisporus* in the world market. Mushroom cultivation is the fifth largest agricultural sector in China with 24 billion USD value and 10% growth rate every year for

the last 30 years (Zhang *et al*., 2014). Oysters are naturally found on rotten wood material. The growing and consumption interest of oyster mushroom is increasing largely due to its taste, medicinal and nutritional properties (Garcha *et al*., 1993). There are many types of mushroom that can be cultivated in Kenya but they have started with *Agaricus bisporous* (buttons), *pleorotus* (oyster) *lentininus* (shiitake) and Ganoderma. This range is continuously being expanded as demand grows.

Mushrooms are grown utilizing agricultural wastes for example cereal straws, maize stocks, bean stock, cotton husks, maize cobs, coffee husks, coffee pulp, paper waste, papyrus, water hyacinth, banana fronds, etc. Mushrooms can be grown in unused buildings among them, Go-downs, garages, chicken houses, unused factories etc. Mud thatched houses also create the right climate. Bricks and stone can also be used. Custom built structures with air conditioning can be cultivated but they are expensive and beyond the ability of many farmers (Mandeel *et al*., 2005).

Although mushroom have been produced and consumed for centuries, the published data concerning chemical composition in general and nutritional value in particular are very limited. In fact there have been no animal feeding studies to estimate the mushroom protein nutritive value (Kalac and Svoboda, 2000; Zhang *et al*.,2014). Royse and Schisler 1980 mentioned that food analysis has tended to dismiss mushrooms as unworthy of serious consideration, as a dietary factor in nutrition. It is evident from the lack of published data that information concerning the chemical analysis and nutritional value of mushroom protein and trace elements would be extremely helpful in evaluating the use of mushroom as a menu or human dietary supplementation. Different mushrooms have different nutrition values but generally they are rich in protein, fibre and vitamins and very low in cholesterol and fats and are therefore referred as health food (Mallikarjuna *et al*.,2013; Mattilla *et al*.,2001).

Determination of bioactive compounds content directly from foodstuff is not enough for the prediction of potential in vivo effects, as metabolites reaching the blood system may be different from the original compounds found in food, as a result of an intensive metabolism that takes place during absorption. Nutritional efficacy of food products may be ensured by the determination of bio accessibility, which provides valuable information in order to select the appropriate dosage and source of food matrices(Lubeka *et al*., 2020).However, between all the methods available, there is a need to establish the best approach for the assessment of specific compounds. Comparison between in vivo and in vitro procedures used to determine bio accessibility and bioavailability is carried out, taking into account the strengths and limitations of each experimental technique, along with an intensive description of actual approaches applied to assess bioaccessibility of bioactive compounds (Nawiri *et al*,. 2013; John *et al*., 2019). However, when studying the role of bioactive compounds in human health, their bioavailability is not always well known. Before becoming bioavailable, they must be released from the food matrix and modified in the gastrointestinal (GI) tract.

Therefore, it is important before concluding on any potential health effect, to analyze whether the digestion process affects bioactive compounds and their stability, as this, in turn, will affect their bioavailability and their possible beneficial effects. Different digestion models have been developed by the scientific community that accurately mimics the complex physicochemical and physiological conditions of the human GI tract, along with in vivo models in living organisms (Hur *et al*., 2011). However, comparison of results between different studies is difficult to accomplish, as there is no defined experimental model for studying bio accessibility and

bioavailability. The present study was carried out to evaluate the nutritive value of mushrooms through the determination of protein and trace element levels in mushroom.

2. Experimental

2.1.1 Sampling and preparation

Mushroom farming in Kenya is a relatively small, and the information on mushroom cultivation in the country is limited. However, the mushroom industry in the country is rapidly growing, and production cannot currently meet an increasing local demand. Local farmers rely on the importation of mother cultures from outside Africa for spawn production. Some farmers also prefer importing spawn instead of mother cultures for cultivation. The imported mush-rooms lineages are faced with numerous challenges including poor regional adaptability, increased susceptibility to pests and diseases and low yields. Exploitation of native strains of mushroom species is therefore likely to provide strains with desirable characteristics for commercial cultivation. Characterization and identification at the species level is an important first step in systematic exploitation of any fungal strain in specific applications. The traditional phenotypic approach is still being used; however, this method has been criticized widely for its high subjectivity to environmental conditions (Jang *et al*., 2003). Characterization with molecular tools such as the AFLP markers and ribosomal DNA (rDNA)internal transcribed spacer (ITS) sequences has proven to be more reliable in biodiversity studies. Different mushroom lineages, including members belonging to the genus Pleurotus have been successfully discriminated with molecular tools (Meng *et al*., 2003).

The four oyster *pleorotus* mushroom varieties: *Pleorotus ostreatus* (PO), *Pleorotus sajor caju* (PS), *Pleorotus pulmonaries* (PP), *Pleorotus cintropiletus* (PC) were collected from farms near Jomo Kenyatta University of Agriculture and Technology (JKUAT) and identified by trained personnel from National Museum of Kenya. The different species of mushrooms were individually cleaned, cut into pieces and placed in a labeled special glass container. Freshly harvested mushrooms were dried in shade for four days, powdered and stored awaiting analysis.

2.1.2 Determination of minerals

Each mushroom sample was air-dried at 105 °C overnight which is different from shade drying and crushed using a mortar and pestle. 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 about 10-15 min. After cooling, 50 ml of deionized water was added and filtered. The amounts of Fe, Zn, Mn, Cu, Cr, Cd, Co, Ni, and Pb were determined using an atomic absorption spectrometer while K, Mg, Na and Ca was determined by flame photometry. Calibration curve procedure was used to determine the concentrations where a series of thirteen standard solution for each element were prepared from a stock solution of 1000 ppm. All analyses were done in triplicates.

2.1.3 Determination of Crude Protein

Protein content of the samples was determined by the use of the macro-kjeldahl method where the content was first determined and multiplied by 6.25 coefficient according to (Osborne and Voogt, 1978., AOAC, 2006) procedure. To determine the protein content, 1.0g of the dry powdered mushroom sample was digested with 5 ml of concentrated tetra-oxosulphate (VII) acid, to which a tablet of selenium catalyst was added in a fume cupboard. The digest was made up to the mark in a 250 ml volumetric flask. Ten ml of the

digest was distilled and titrated with 0.2N H_2SO_4. The crude protein was equaled to the N multiplied by a conversion factor, 6.25.The protein content was calculated by multiplying the total nitrogen by a conversion factor 4.38 based on the presence of 70% of digest able protein (Barroetaven˜a, and Toledo, 2017)

2.1.4 Method validation for trace elements

Calibration curves of peak areas against concentration of Fe standards were plotted (Figure 1.0). The curves for Fe were linear within the concentration range determined.The calibration line for Fe gave a correlation coefficient $r^2 = 0.999$ and regression equation y = 0.007x .The regression equation and correlation coefficient of the rest of the trace element are as shown in Table 1.1.

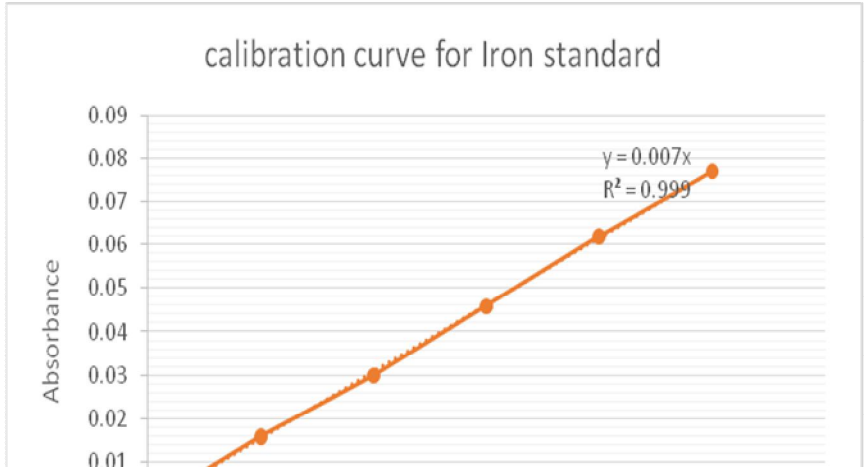

Figure 1.0 Calibration curve for Iron standard

Table 1.1 of regression equations and R values

Elements	Regression Equation	R^2 Values
K	Y=1.1X -0.33	0.975
Ca	Y=0.007+0.002	0.974
Mg	Y=0.096-0.011	0.986

Fe	Y=0.007X	0.999
Cu	Y=0.012X+0.001	0.995
Zn	Y=0.075X+0.010	0.992
Cd	Y=0.041X-0.001	0.995
Ni	Y=0.02X-0.000	0.974
Cr	Y=0.004X-0.000	0.989
Na	Y=0.985+1.823	0.997
Mn	Y=0.012X	0.992
Co	Y=0.005X	0.988
Pb	Y=0.004X-0.000	0.995

3. Results and discussion

3.1 Elements composition

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).In this study the results obtained are shown in Table 1.2. These results are comparable to those in literature (Rudawska and Leski, 2005; Aletor, 1995).

Table 1.2: Concentration of Trace elements in the four Oyster mushrooms in mg/kg

Mushroom	K	Na	Mn	Co	Pb	Zn	Cd	Ni	Cr	Cu	Fe	Ca	Mg
PS	60.909	26.891	0.639	1.46	0.948	0.047	0.165	1.966	1.561	0.167	1.169	3.592	1.579
PO	73.030	23.510	0.694	1.127	1.170	0.060	0.181	1.851	1.485	0.325	1.905	0.869	1.589
P C	73.030	24.524	0.639	1.127	1.170	0.059	0.173	1.966	1.636	0.299	1.861	0.822	2.277
PP	54.848	23.848	0.611	1.193	1.244	0.048	0.173	2.195	1.333	0.352	1.299	0.869	1.932

K, P, Na and Mg constitute about 56 to 70% of the total ash content of the mushrooms while potassium alone forms 45% of the total ash. Abou-Heilah *et al*., (1987) found that content of potassium and sodium in Agaricus bisporous was 300 and 28.2 ppm respectively. Agaricus. bisporus ash analysis showed high amount of K, P, Cu and Fe (Zhang,2014) reported that M. esculenta contains Ca (0.5776 mg), P (3.313 mg), Fe (1.213 mg) and K (3.831 mg). Varo *et al*., (1980) reported that *Agaricus bisporus* contains Ca (0.04 g), Mg (0.16), P (0.75 g), Fe (7.8 g), Cu (9.4 mg), Mn (0.833 mg) and Zn (8.6 mg) per kilogram fresh weight. Mushrooms have been found to accumulate heavy metals like cadmium, lead, arsenic, copper, nickel, silver, chromium and mercury (Mejstrick and Lepsova, 1993; Wondratschek and Roder, 1993; Kalac and Svoboda, 2000; Svoboda *et al*., 2001; Issilogglu *et al*., 2001; Malinowska, 2004). 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 content of mineral elements (Ca,K,Mg,Na,P,Cu,Fe,Mn,Cd,Pb and Se) have been determined in study of the cultivated mushrooms Agaricus bisporous/brown, Lentinus edodes and pleurotus ostreatus. Cultivated mushrooms were found to be good sources of many minerals eg the content of K, P, Zn and Cu varied in the ranges 26.7-47.3g/kg,8.7-13.9g/kg,47-92g/kg and 5.2-35mg/kg dw respectively (Caglarimak, 2009). Copper levels in mushrooms reported in literature are 4.71-51.0 (Varo *et al.*,1980), 13.4-50.6 mg/kg(Svoboda *et al.*,2001) and 12-181mg/kg(Soylak *et al.*,2005).The values recorded are comparable to those found in this study. Levels of Fe reported in literature were 146-835mg/kg (Sanchez,2010) 31.3-11.90mg/kg (Soylak *et al.*,2010) and 180-407mg/kg(Mallikarjuna *et al.*,2013)The lowest and the highest level of Mn present in studied mushrooms were 0.02mg/kg and 0.04mg/kg respectively. The minimum and maximum levels of Zn present in the studied wild and cultivated samples were below the permissible limit of 60mg/kg of zinc in foods (Caglarimak,2009). Zinc levels reported in literature were 45.2-173.8mg/kg(28),33.5-89.5mg/kg(Aletor,1995) and29.5-158mg/kg(Mattilla et al.,2001).The values recorded in the study are lower compared to other studies can be attributed to various factors such as analytical methods used and the substrate on which they were cultivated.

Protein

In terms of the amount of crude protein, mushrooms rank below animal meats but well above most other foods including milk (Chang, 1980). On a dry weight basis, mushrooms normally contain 19 to 35% proteins as compared to 7.3% in rice, 12.7% in wheat, 38.1% in soybean and 9.4% in corn (Crisan and Sands, 1978; Bano and Rajarathnam, 1988). Table 1.2 shows the composition of four mushroom types. The protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms and the substrate on which the mushroom is grown (Zied *et al.*,2019;Bano and Rajarathnam, 1982). In this study the four mushroom protein levels were as follows pleorotus ostreatus (36.86%), Pleurotus pulmonaries (33.49%), Pleurotus cintropileatus (35.05%) and Pleurotus saju caju (36.68%). The values obtained are comparable to those reported by Singh and Chaube 1995 who reported a protein content of edible mushrooms on dry weight basis to be as follows pleurotus ostreatus (27.4%), Pleurotus florida (37.19%), Pleurotus sajor caju (36.94%). Obodai *et al.*, 2009 reported that in general the fruiting bodies of mushrooms contain about 25% protein.

Table 1.3 Protein content of four oyster mushrooms

Sample code	% CP
PO	36.86
PS	36.68
PP	33.49
PC	35.05

CONCLUSION

On a dry weight basis, Oyster Mushrooms have substantial protein, which is above 35 %. The variation in the reported protein nutritional analysis value for protein in oyster mushrooms from what is in literature which ranges from 15-35% is due to several factors. The protein content is affected by the type of substrate and by the spawning media and rate.Finally strains of Pleurotus vary in their nutritional composition and yield performances. 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. The mineral content of wild edible mushrooms has been found higher than cultivated ones .The concentration range for trace elements were comparable to those in literature

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