Evaluation of Antioxidant and Iron Chelating Activities of A Wild Edible Oyster Mushroom *Pleurotus Cystidiosus* from Tanzania

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Abstract
This study was conducted in order to assess the levels, activities and establish the influence of postharvest storage methods on antioxidants of the giant wild edible mushroom *Pleurotus cystidiosus.* Standard analytical methods were employed to determine the level and activities of antioxidants. It was followed by comparing the results in order to establish the influence of postharvest storage methods namely heat drying, freezing, sun drying, refrigeration, salt drenching and dehydration using silica gel beads. The mushroom caps and stipes were treated separately. Results showed that irrespective of the preservation method and portion of the mushroom used, *P. cystidiosus* exhibited very high values of the number of antiradical activity units in 1 mg of extract (EAU_{515}), ranging from EAU_{515} 3.25 to 17.00. The mushroom also portrayed very high 1,1-diphenyl–2picryhydrazyl (DPPH) free radical scavenging ability recorded at very low concentrations of crude extracts (0.0125 to 0.800 mg/ml) and the EC_{50} values ranging from 0.035 to 0.150 mg/ml. Likewise the highest ferrous iron chelating ability of 98.3% was recorded at a low concentration of 0.80 mg/ml. With respect to the antioxidant activities in relation to the fruiting body portions, there were consistently higher antioxidant levels and antioxidant activities in the cap portion than in the stipe of the mushroom. Preservation method also influenced the antioxidants activities whereby the highest radical scavenging and iron chelating activities were found in the oven dried, silica gel dried and refrigerated mushrooms while, salt drenched mushrooms performed the least. These results suggest that *P. cystidiosus* contain antioxidant levels and activities which are useful properties for a valuable food source of nutraceutics potential; however, salt drenching method of preservation should be avoided. This giant mushroom is recorded for the first time in the country thus, isolation of its germplasm for spawn production, and subsequent domestication remains to be investigated.
Keywords: Antiradical scavenging, iron chelating, antioxidant, wild *Pleurotus cystidiosus*

1. Introduction

Mushrooms have been widely consumed world over as rich food (Gbolagade *et al.* 2006, Kalac 2009). But also as medicine for treating various diseases including cancer, high blood pressure, heart ailments and many others (Chang 1996, Achrya 2007). More recent studies (Gregori *et al.* 2007, Kim *et al.* 2009, Pal *et al.* 2010, Adebayo *et al.* 2012, Tibuhwa 2012) have shown the importance of mushrooms as functional food, because they have a variety of accumulated secondary metabolites such as phenolic compounds, polypeptides, terpenes, and steroids. A variety of the mushrooms phenolic compounds are known to be excellent anti-oxidants due to their ability to neutralize excess radicals which are reactive oxygen species in cells. These radicals are constantly produced in the human body. Thus mushroom consumption helps the endogenous cell defense system to reduce oxidative damage (Temple 2000, Fang *et al.* 2002). Iron chelators are among the important free radical scavengers found in some mushrooms (Pal *et al.* 2010). They play an important role in detoxifying metal ions and prevent poisoning.

There is a long tradition by many tribes in Tanzania of using wild mushrooms as food and medicine (Harkonen *et al.* 2003, Tibuhwa 2012). Among the common widely used mushrooms genera in Tanzania are *Pleurotus* (oyster mushroom) *Agaricus, Termitomyces Cantherellus* and *Lactarius. Pleurotus* species, commonly known as oyster mushrooms are highly nutritive with gastronomic nutritional and medical properties and can easily be cultivated on large range of substrates (Khanna and Gareha 1984, Adebayo *et al.* 2012). In Tanzania three *Pleurotus* species namely *P. flabelatus, P.sajor–caju and P. citrinopileatus* are domesticated/cultivated and commonly found in the food markets. Recently a giant edible oyster mushroom *P. cystidiosus* commonly found in Dar es Salaam and other coastal areas of Tanzania, growing on the logs of trees, producing enormous fruiting bodies with one fruiting body weighing up to one kilogram (Figure 1) was collected. The sheer size of the fruiting bodies and the fact that it is very popular as food among the community where it is found has generated a lot of study interest on its taxonomy and nutritional potential. In this study, its antioxidant values and activities, and whether different preservation methods pose any effect on their values are investigated.

Figure 1. *Pleurotus cystidiosus* fruiting bodies in the field at Mwalimu J.K.Nyerere Mlimani Campus, University of Dar es Salaam. Photo taken by D. Tibuhwa in March 2014

2.0 Materials and Methods

2.1 Sample collection

Fresh samples of mushroom identified as *Pleurotus cystidiosus*, were collected from a log of a *Ficus benjamina* tree at the Mwalimu J.K.Nyerere Mlimani Campus, University of Dar es Salaam during early rains of March 2014. Field observation and morphological characterization including spore printing were done. The sample lot was apportioned and subjected to different preservation treatments namely oven heat drying at 40^0C, freezing at -20^0C, sun drying for seven days, refrigeration at 4^0 C, salt drenching and dehydration using silica gel beads. The mushroom caps and stipes were treated separately.

2.2 Crude extract preparation

Methanolic extractions were carried out according to Tibuhwa *et al.* (2012) whereby 1gm of mushroom cap or stipe from each preservation treatment was weighed and crashed using motor and pestle, then soaked in 250 ml of methanol. Extraction was done according to the method by Pal *et al*. (2010). The soaked material was constantly stirred for 48 hours, thereafter filtered using whatman filter paper and evaporated to dryness at 40 ^{0}C under reduced pressure, in a rotary evaporator with 90 rpm. The obtained concentrates were stored in dark at 4 ^{0}C until further use. The percentage yield of the evaporated extracts was calculated based on dry weight (Maisuthisakul *et al.* 2007) as shown below:

$$Yield\ (\%) = \left(\frac{W1}{W2}\right) * 100$$

Where: W_1 = weight of the extract after methanol evaporation,
W_2 = dry weight of the mushroom sample

2.3 Measurement of antiradical activity

Measurement of antiradical activity is based on the principle that DPPH (1,1-difenly-2-picrylhdrazyl) radical in its form has a characteristic absorbance at 515 nm, which disappears after its reduction by an antiradical compound. Thus the reduction of DPPH can be monitored using spectrophotometer by measuring decrease in its absorbance at 515 nm during the reaction method as described by Brand-Williams *et al.* (1995).

In this study, 40 µl of methanolic extract were added to 1460 µl of 0.0037% DPPH. Absorbance was measured at 515 nm at time 0 and after 1 minute. The antiradical activity unit (AU_{515}) of each extract was calculated according to the equation:

$AU_{515} = (A_0 - A_1) - (A_{0C} - A_{1C})$; where

AU_{515} = Antiradical activity of the extract,

A_0 = Absorbance of the sample at the beginning of the reaction (0 minute),

A_1 = Absorbance of the sample after 1 minute of the reaction,

A_{0C} = Absorbance of the control sample at the beginning of the reaction

A_{1C} = Absorbance of the control sample after 1 minute of the reaction.

The number of antiradical activity units (EAU_{515}) per 1 mg of each extract was calculated according to the equation:

$$EAU_{515} = \frac{AU_{515}}{le}$$

where:

EAU_{515} = number of antiradical activity; le = amount of extract in the simple (mg) and AU_{515} = antiradical activity of the extract.

The total number of antiradical activity units in the extract was calculated according to the equation:

$$PAU_{515} = \frac{cle * AU_{515}}{le}$$

where:

cle = total number of extract (mg) and le = amount of extract in the sample (mg).

2.4 DPPH free radical scavenging activity

The 1, 1-diphenyl–2picryhydrazyl (DPPH) free radical scavenging activity was determined according to Masuda *et al.* (2000) and Pal *et al.* (2010). The method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger. A dilution series of methanol crude extracts solutions (1:10- 1:10^7) were prepared. One ml of the extract was mixed with 1 ml of 0.4 $mmol^{-1}$ methanolic solution containing DPPH. The mixture was left in the dark for 30 min and the absorbance measured at 515 nm. The percentage of DPPH radical scavenging activity of each extract was determined at these seven concentrations and was calculated as:

$$\text{DPPH radical scavenging activity} = \left[\left(A_0 - \left(\frac{A_1 - A_S}{A_0} \right) \right) * 100 \right]$$

Where A_0= Absorbance of the control solution containing DPPH only

A_1= Absorbance in the presence of mushroom extract in DPPH solution

A_s= Absorbance of the sample extract solution without DPPH

Total antioxidant necessary to decrease the initial DPPH radical concentration by 50% (EC_{50} value) was determined from the plotted graph of scavenging activity against the concentration of extracts.

2.5 Chelating ability of ferrous ions

Chelating ability was determined according to the method of Dinis *et al.* (1994). Different concentration of each extract (1:10 to 1:10^6) in water (1 ml) were mixed with 3.5 ml of methanol and 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by the addition of 2 ml of 5mM ferrozine. After 10 minutes at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. EC_{50} value (mg/ml extract) which is the effective concentration at which ferrous ions were chelated by 50% was obtained by interpolation from linear graph of chelating activity against the concentration of extracts.

2.6 Determination total phenolic content

Phenolic content of the mushroom methanolic extracts was determined using Folin-Ciocalteu reagent described by Singleton *et al.* (1999). 0.1 g of each of the concentrated extracts was diluted with 5ml ethanol from which 200 µl were transferred into a test tube, then mixed thoroughly with 1 ml Folin–Ciocalteu reagent for three minutes. Then 0.8 ml of 7.5% sodium carbonate was added and the mixture was agitated for 30 min in the dark, followed by centrifugation for 5 minutes at 3300 g. Absorbance of the mushroom samples and a prepared blank were measured at 765 nm using spectrophotometer (Uv-vis model 6305 Jenway UK). The concentration of total phenolic compounds in the extracts was expressed as milligram of Gallic acid equivalents (GAE) per 100 g weight of mushroom using linear equation obtained from the standard Gallic acid calibration curve (Tibuhwa *et*

al. 2012).

2.7 Total Flavonoid Content

Total flavonoid content was determined according to Pal *et al.* (2010) using quercetin as standard. One (1 ml) of each mushroom extract was diluted with 4.3 ml of 80% aqueous ethanol containing 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate and left to stand for 40 minutes at room temperature (give the temperature range since room temperature is relative). The absorbance was determined spectrophotometrically at 4.5 nm using Uv-vis model 6305 Jenway UK. Total concentration was calculated using quercetin standard curve, and expressed as Rutin equivalent/100 g of mushroom extract.

2.8 Determination of Vitamin C

The vitamin C content was determined by titration using 2,6 Dichloropheno indophenols methods described by Plummer (1987). Known weight of ground sample was mixed with 25 ml of 5% metaphosphoric acid solution and shaken for 30 min. The mixture was then filtered with Whatman no. 42 filter paper using suction pump. 10 ml was pippeted from the extract into 250 ml conical flask and titrated against 0.025% of 2,6 Dichlorophenol Indophenol reagents. The amount of vitamin C in each extract was calculated from following equation:

$$\text{mg of ascorbic acid per } 100\text{ g} = \frac{(A * I * V_1 * 100)}{(V_2 * W)}$$

Where: A = quantity of ascorbic acid (mg) reacting with 1 ml of 2, 6 Indophenol
I = volume of Indophenol (ml) required for completion of the extract titration
V_1 = total volume of extract
V_2 = Volume of aliquot
W = weight of ground mushroom

2.9 determination of β – Carotene and Lycopene Contents

β-**C**arotene and Lycopene contents were measured using the method of Nagata and Yamashita (1992). One hundred (100) mg of a mushroom extract was shaken with 10 ml of Acetone-hexane mixture (92:3) for 1 minute and filtered through Whatman number 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. The β – carotene and lycopene content were calculated as:

Lycopene µg/mg $= 0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$

β–carotene µg/mg $= 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$

3.0 Results and Discussion

3.1 Crude extract % yield

Results showed significant variations in crude extract yield among the different preservation methods and between the cap and the stipe parts of the same mushroom (Figure 2).

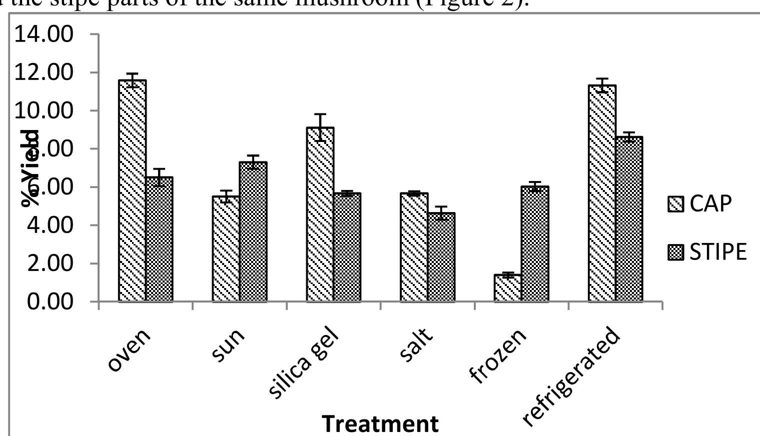

Figure 2.Percentage yield of extracts from mushroom samples.
Values are means of three different measurements (Mean±SD, n=3)

In the overall, highest yields were obtained in the cap portions preserved by oven drying (11.59%), followed by refrigeration (fresh) and silica gel drying. Lowest yields in the cap portions were recorded in frozen samples (1.40%), sun dried (5.51%) and salt drenched (5.68%) samples. With the exception of frozen and sun dried samples, crude extract yields were consistently higher in the cap than in the stipe portions. Very low yield observed in frozen samples could be associated with significant losses of mushroom content experienced during defrosting process of the cap samples.

3.2 Antiradical activity

The results of antioxidative properties of the mushroom *Pleurotus cyctidiosus* subjected to different preservation methods are presented in Table 1. Remarkable differences in the antiradical activity units (AU_{515}), number of antiradical activity units in 1 mg of extract (EAU_{515}) and number of antiradical activity units in the extract (PAU_{515}) were observed between the cap and stipe portions, being consistently higher in caps than in stipes. Of all the preservation methods used, the highest EAU_{515} and PAU_{515} were shown by sun dried (EAU_{515} 17.0; PAU_{515} 936.7) followed by silica gel dried (EAU_{515} 14.8; PAU_{515} 1,345.2), and finally by salt drenched and refrigerated cap samples. EAU_{515} values of *Pleurotus cystidious* reported in this study are by far much higher than those reported on *Termitomyces* species by Tibuhwa (2012). These differences could have been brought about by inherent differences in the distribution of nutrients resources in different mushroom species and mushroom parts, which may be influenced by the type and nature of substrate used for growth. In this case while *Termitomyces* are symbiotic mushrooms which grow on termite mounts, *Pleurotus cyctidiosus* is saprophytic, growing on dead logs of hard wood.

Table 1. Antiradical activities of mushroom samples preserved in various conditions
(Values are means of three different measurements)

Preservation method	**Part of the mushroom**	**% Yield**	**Antiradical activity units (AU_{515})**	**Number of antiradical activity units 1 mg of extract (EAU_{515})**	**Total number of antiradical activity units in the extract (PAU_{515})**
Oven dried	Cap	11.59±0.36	0.191±0.01	4.75	550.25
	Stipe	6.51±0.45	0.211±0.01	5.25	341.80
Sundried	Cape	5.51±0.31	0.677±0.02	17.00	936.70
	Stipe	7.31±0.35	0.146±0.02	3.75	274.80
Silica gel dried	Cap	9.12±0.70	0.586±0.03	14.75	1,345.20
	Stipe	5.67±0.13	0.089±0.01	2.25	127.60
Salt drenched	Cap	5.68±0.12	0.433±0.01	10.75	610.60
	Stipe	4.64±0.34	0.131±00	3.25	150.80
Frozen (-20^0C)	Cap	1.40±0.13	0.331±0.00	8.25	115.50
	Stipe	6.04±0.24	0.424±0.01	10.50	634.20
Refrigerated (4^0C)	Cap	11.36±0.36	0.268±0.01	6.75	764.80
	Stipe	8.62±0.24	0.222±0.00	5.50	474.80

3.3 DPPH free radical scavenging ability

The DPPH radical scavenging ability of all mushroom extracts increased with increasing concentrations of the extracts from 0.0125 to 1.0 mg/ml (Figure 3). Of all the samples, mushroom caps preserved by oven drying, refrigeration and silica gel drying showed highest radical scavenging activity at all concentrations, respectively. At a concentration of 0.8 mg/ml, the radical scavenging activities were 89.3, 86.6 and 81.1% in the same order. Generally, mushroom stipe samples showed lower radical scavenging ability than mushroom cap samples.

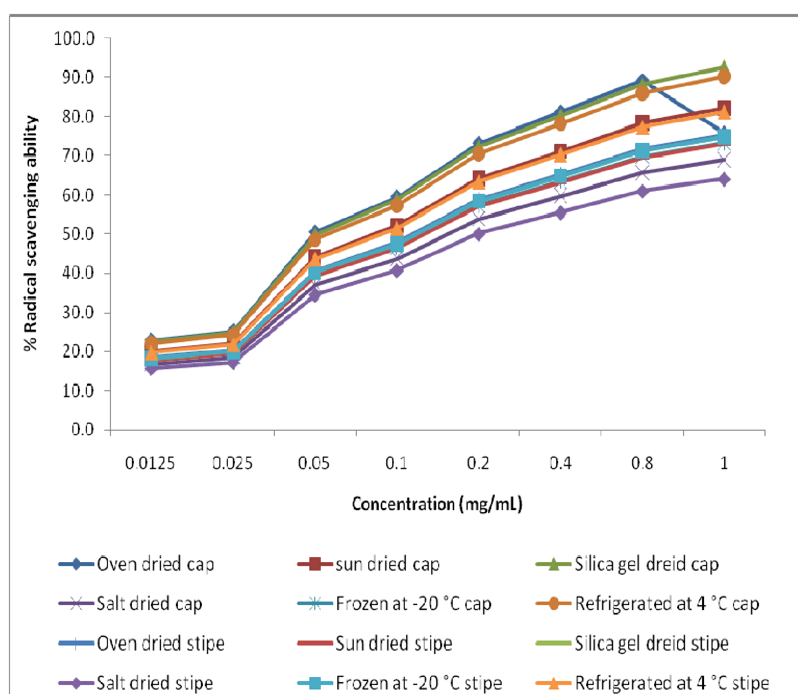

Figure 3. DPPH of mushroom cap and stipe stored under different preservation conditions

Previous studies by Kim *et al.* (2009) have shown radical scavenging activities of oyster mushrooms at 2 mg/ml concentration to be 84.4, 54.5 and 29% for yellow, pink and dark-grey mushroom strains, respectively. Mau *et al.* (2002) and Lo (2005) reported similar values at 6.4 mg/ml (81.8%) and 5 mg/ml (68.4%), respectively. Studies by Tibuhwa (2012) reported scavenging activity values at concentration of 1 mg/ml for *Termitomyces microcarpus* (98.65%), *T. letestui* (95.95%) and *T. titanicus* (62.14%). Results from this study have shown higher radical scavenging ability of *Pleurotus cystidiosus* at lower concentrations of 0.2 to 1.0 mg/ml. The total antioxidants required to decrease the initial DPPH radical concentration by 50% (EC_{50} value) as determined from the plotted graph of scavenging activity against different concentrations of the extracts are presented in Table 2. Higher scavenging abilities are well known to help in protecting the endogenous system by scavenging the free radicals that play a major role in the wide ranges of pathological disturbances (Halliwell 1984, Tibuhwa 2012). These results thus, show that this mushroom has a potential of being developed into a nutraceutics. It also calls for the need of domesticating and including it into people's regular diets.

Table 2. Radical scavenging ability – EC50 values (mg/ml) of mushroom cap and stipe samples

	Cap (mg/ml)	Stipe (mg/ml)
Oven-dried	0.0359	0.0828
Sundried	0.0594	0.0922
Silica gel dried	0.0367	0.0844
Salt drenched	0.1094	0.1500
Frozen (-20^{0}C)	0.0906	0.0844
Refrigerated (4^{0}C)	0.0406	0.0656

The EC_{50} values in mg/ml of oven dried (EC_{50} 0.0357), silica gel dried (EC_{50} 0.0367) and refrigerated (EC_{50} 0.0406) mushroom caps were lowest, signifying that they display a very high scavenging ability. Salt drenched mushroom cap and stipe had the highest EC_{50} values of 0.1094 and 0.1500 mg/ml respectively showing low radical scavenging ability. Regardless of the mushroom preservation method used, the free radical scavenging ability *of Pleurotus cystidiosus* reported in this study is by far better than most of the reported antiradical mushrooms. Tibuhwa (2012) reported super radical scavenging ability of termitarian mushrooms as EC_{50} 0.1, 0.14 and 0.36 mg/ml for *Termitomyces microcarpus, T. letestui* and *T. eurhizus,* respectively. Study by Filipia *et al.(2010)* reported EC_{50} values higher than 0.5mg/ml where as Pal *et al*. (2010) recorded EC_{50} between 0.340 to 1.500mg/ml. In overall, the radical scavenging ability (EC_{50}) by *Pleurotus cystidiosus* reported in this study is 3 to 16 times far better (lower) than those reported in literature.

3.4 Iron chelating ability

Iron chelators are small molecules that bind tightly to metal ions. The key property of all chelators is that the

metal ion bound to the chelator is chemically inert. Thus one of their important roles is to provide protection of our bodies by removing excess iron from the blood hence prevent poisoning. The principle and trend in the iron chelating ability was similar to that of the DPPH radical scavenging. Ferrozine quantitatively forms complexes with Fe^{2+}. In the presence of chelating agent, the complex formation is disrupted, thus resulting in the reduction of the red colour (Pal *et al.* 2010). Reduction therefore allows estimation of chelating ability of the mushroom extract.

Iron chelating ability of *P. cystidiosus* extracts increased with the concentration of extracts from 0.025 to 0.40 mg/ml but also varied with the method of preservation and the part of mushroom, cap or stipe (Figure 4). The highest iron chelating activities at a concentration of 0.40 mg/ml were observed in extracts from oven dried sample (98.3%) followed by silica gel dried sample (96.9%) and refrigerated sample (94.7%). The least chelating capabilities were shown by salt drenched cap and stipe samples. Generally, the stipe had lower iron chelating ability than the cap. This trend very much mimics the DPPH radical scavenging activities. Chelating effects on ferrous ion from methanolic extracts from other mushrooms have been reported. For example *Pleurotus citrinopileatus* have been reported by Lee *et al*. (2007) to chelate ferrous ions by 82.1% at 5mg/ml. Lo (2005) reported chelating abilities of *Pleurotus eryngii, P. ferulae* and *P. ostreatus* at a concentration of 5 mg/ml to be 41.4 to 64%. Similarly, Kim *et al.* (2009) reported higher chelating abilities (78-80%) of coloured oyster mushrooms at even lower concentration of 1.5 mg/ml. In this report, the studied mushroom, *Pleurotus cystidiosus* exhibited higher chelating abilities by all the crude extracts (67.1 – 98.3%) at a very low concentration of 0.4 mg/ml, than so far reported. This is an indication that the mushroom studied is a very beneficial food source, with powerful antioxidant abilities.

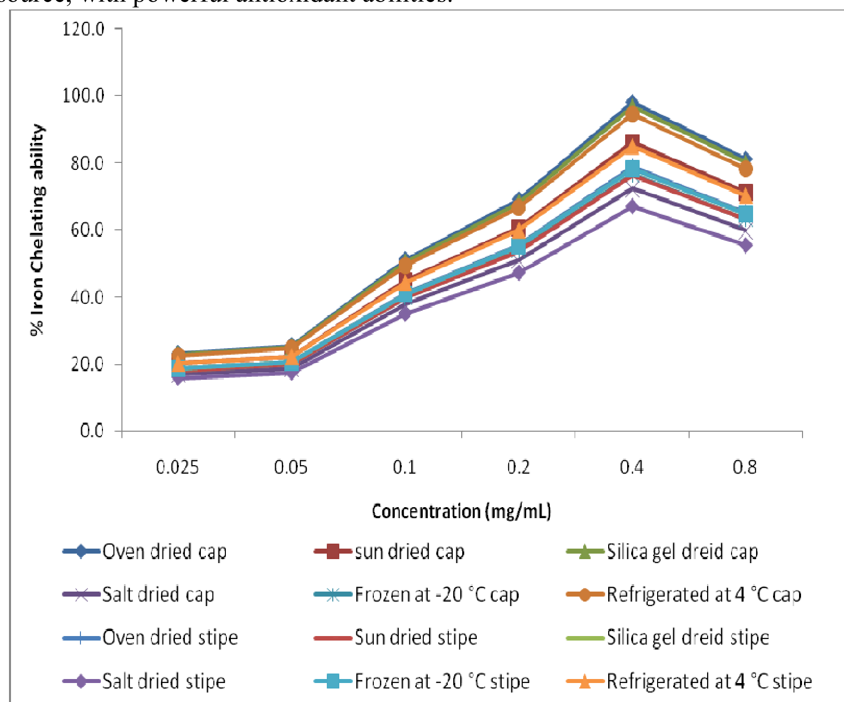

Figure 4. Ferrous iron chelating ability of extracts from *Pleurotus cystidiosus* cap and stipe

The EC_{50} values of iron chelating abilities (Table 3) were lowest in the extracts from oven dried samples (0.0911mg/ml) followed by silica gel dried samples (0.0962 mg/ml) and refrigerated samples (0.1054 mg/ml). Salt drenched samples had the highest EC_{50}: 0.2270 mg/ml for the stipe and 0.1919 mg/ml for the cap, implying that the iron chelating capability was much reduced.

Table 3. Ferrous iron chelating ability – EC_{50} values of *P. cystidious* cap and stipe

	Cap (mg/ml)	Stipe (mg/ml)
Oven-dried	0.0911	0.1622
Sundried	0.1325	0.1703
Silica gel dried	0.0962	0.1622
Salt drenched	0.1920	0.2270
Frozen (-20^{0}C)	0.1703	0.1378
Refrigerated (4^{0}C)	0.1054	0.1622

In the overall, mushroom preservation methods used prior to preparation of crude extracts had an influence on the antiradical and iron chelating activities of the extracts. While sun-drying, silica gel drying and refrigeration

had best activity values, salt drenching and frozen samples performed least. It is also evident from the results that higher antioxidant and iron chelating activities were found in the cap portions of the mushroom than in the stipe. These molecules may naturally be fewer in the fibrous stipe than in the cap portions, or the methanolic extraction method was not suitable for removing out the molecules from the fibrous stipe material.

3.5 Phenolic and flavonoid content

Mushrooms contain various polyphenolic compounds recognized as excellent antioxidants not only because of their ability to scavenge free radicals by single electron transfer (Mau *et al.* 2004, Cheung *et al*. 2003) but also because of their stable radical intermediates (Shahidi and Wanasundra 1992). In the present study, phenolic content expressed in mg gallic acid equivalent per100g mushroom extract differed among the different preservation methods and between the mushroom cap and stipe (Figure 5). Very high phenolic content (99.26 mg GAE/100g) was observed in the mushroom caps preserved under salty condition than all other methods which had more or less similar values. These values are higher or comparable with those reported on mushroom by Pal *et al*. (2010), Maisuthisakul *et al*. (2005) and Batool *et al.* (2010) on plants. However, Kim *et al.* (2007), Tibuhwa (2012) and Adefegha and Oboh (2011) reported much higher phenolic contents in oyster mushroom, termitarian mushrooms and leafy vegetables , respectively.

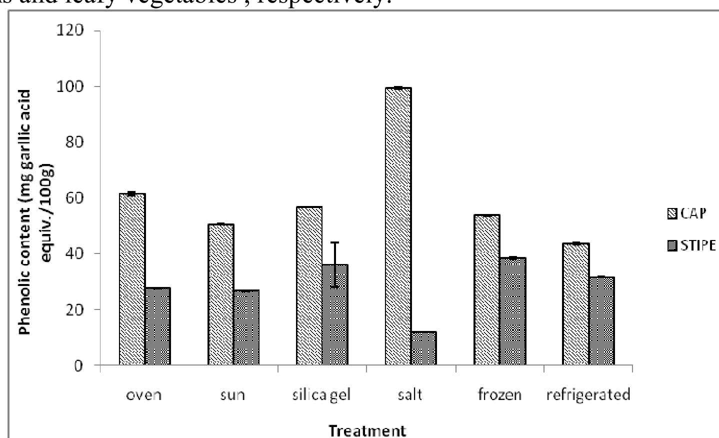

Figure 5. Phenolic content of *Pleurotus cystidious cap and stipe.*
Error bars indicate standard deviation of the means of triplicate readings

Flavonoids are usually glycosilated and are responsible for the orange, red and blue color in fruits and vegetables. Generally deep colored fruits, vegetables or foods including mushrooms are regarded as more healthy to human body (Lin and Tang 2007). Thus a study on the level of these pigment components in mushrooms has attracted interest of many scientists (Kim *et al*. 2009). In this study, flavonoid content expressed in mg Quercetin equivalent per 100 g was highest in the oven-dried mushroom caps (31.64 mg quercetin equiv./100 g), followed by silica gel dried and refrigerated mushroom caps (Figure 6). Comparable values have been reported on by Adefagha and Aboh (2011) on vegetables, Tibuhwa (2012) on termitarian mushrooms; while higher values were reported by Tibuhwa *et al*. (2012) on *Coprinus cinerus*, and Kim *et al* on oyster mushrooms. On the other hand, Pal *et al*. (2010) and Adebayo *et al*. (2012) reported much lower values on *Pleurotus pulmonaris* and *P. squarrosulus*, respectively, than those found in this study.

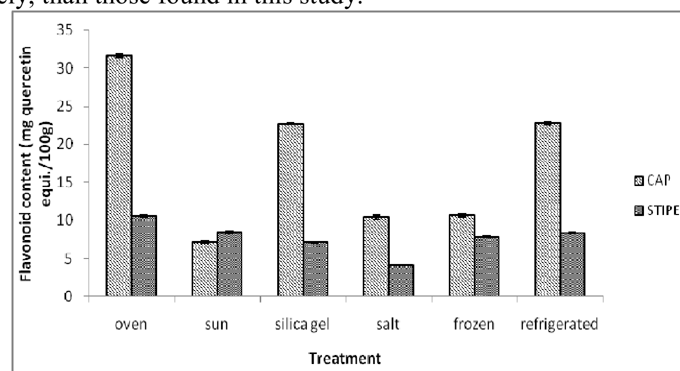

Figure 6. Flavonoid content of *Pleurotus cystidious cap and stipe.*
Error bars indicate standard deviation of the means of triplicate readings

It is evident from the results of this study that mushroom extracts with higher phenolic content did not always have higher flavonoid content. Nevertheless, phenolic and flavonoid content were always higher in the

mushroom caps than in the stipes. The results suggest that the two mushroom parts (cap and stipe) contain different levels of phenolic compounds and flavonoids.

3.6 β-Carotene and lycopene

β-carotene and lycopene are powerful antioxidants and play important roles of enhancing vitamin A in mammals (Pal *et al*. 2010), lowering risk of cancer and heart diseases (Rao and Agarwal 2000). In this report, the levels of β-carotene in the mushroom extracts were influenced by the post-harvest storage in the same pattern. Except for extracts from sundried samples, highest levels were found in silica gel dried samples, followed by salt drenched and frozen cap samples (Figure 7 and Figure 8).

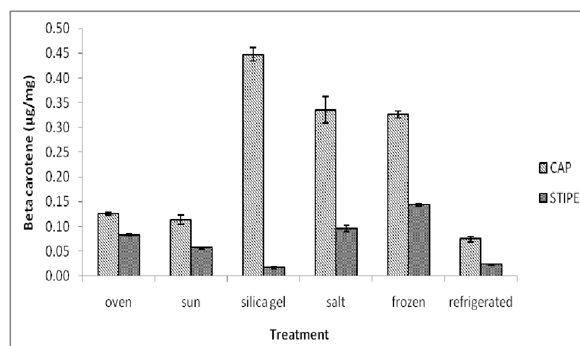

Figure 7. β-carotene content of *Pleurotus cystidiosus* cap and stipe.
Error bars indicate standard deviation of the means of triplicate readings

While β-carotene was lowest in sundried samples, lycopene was highest in same sundried samples. In the overall, appreciable concentrations of β-carotene and lycopene found in this study are comparable to those reported by Pal *et al.* (2010) on *Pleurotus squarosulus* but much lower than those reported by Tibuhwa (2012) on termitarian mushrooms.

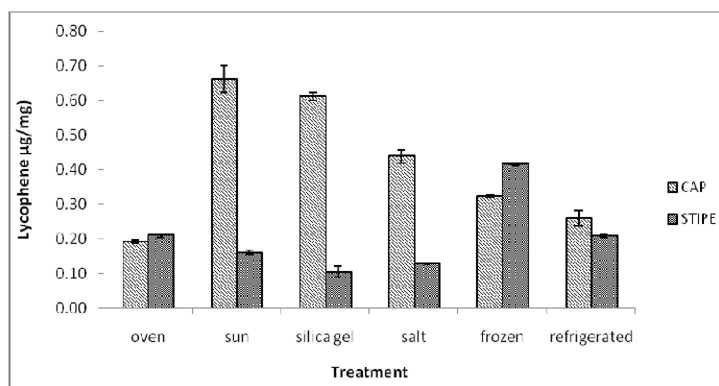

Figure 8. Lycopene content of *Pleurotus cystidious cap and stipe.*
Error bars indicate standard deviation of the means of triplicate readings

3.7 Vitamin C content

Vitamin C is a water soluble antioxidant which is in a unique position to scavenge aqueous peroxyl radicals before these destructive substances damage the lipids in our bodies (Rice-Evans and Miller, 1995). Vitamin C levels of the studied mushroom under different preservation conditions are presented on Figure 9.

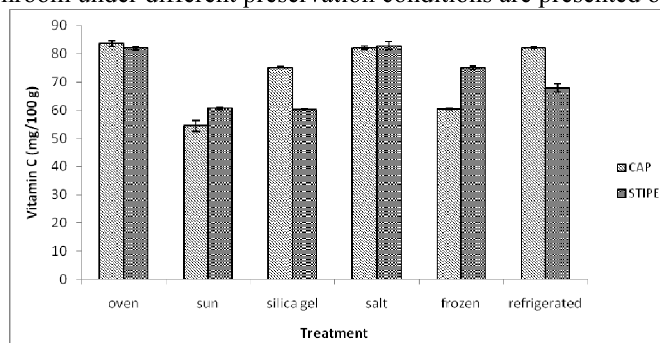

Figure 9. Vitamin C content of *Pleurotus cystidiosus* cap and stipe.
Error bars indicate standard deviation of the means of triplicate readings

Results show that except for the sundried samples, vitamin C values did not vary significantly among the different preservation methods, and between the cap and stipe part of the fruiting body. Vitamin C values ranged between 54.42 and 83.65 mg/100g being lowest in sundried samples. The values were within the range with those reported by Mshandete and Cuff (2007) on 3 mushroom genera (33-55 mg/100g). However, much higher vitamin C contents have been reported recently on mushrooms. For example, Tibuhwa (2012) reported 200-480 mg/100g in termitarian mushrooms and Tibuhwa *et al*. (2012) reported 60-200 mg/100g in *Coprinus cinereus.* It appears drying by exposing mushroom samples on the sun radiation decreases vitamin C content.

4. Conclusion

This study reports for the first time the evaluation of the levels and activities of antioxidants of the wild edible mushroom *Pleurotus cystidiosus*. *P. cystidiosus* has shown uniquely high antiradical scavenging and iron chelating abilities, making it one of the mushrooms with the highest antioxidants properties. Its enormous size, deliciousness and now high antioxidant abilities not only make it a food of the choice, but also envisage its domestication and possible up-scaling and commercialization.

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