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Original Article

Effect of different post harvest treatments on nutritive and antioxidant activities of wild edible *Coprinus cinereus* (Schaeff.) S.Gray from Tanzania

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Abstract

Coprinus cinereus is a wild edible and medicinal mushroom available in Tanzania. Methanolic extracts were prepared from whole young and immature mushrooms and the effect of eight different post harvest treatments on nutritional content, antioxidant component and scavenging activities on DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals were studied. Result showed that *Coprinus cinereus* fruit bodies are good source of antioxidant and proteins although post harvest treatments had profound effects, which varied significantly at ($p<0.05$). The highest scavenging ability (LD 50 = 0.119) was found from mushrooms sun dried for 5 days followed by (LD50= 0.133) recorded from young and immature mushrooms (control) while the least (LD50=4.24) was obtained from mushrooms dipped in boiling 10% salt water for 1minute. On the other hand, crude protein ranged between 7-26% with highest and lowest obtained from oven dried and boiled in 10% salt for 1 minute, respectively. For total flavonoid (mg RE/g) the highest value was obtained from control (44.7) followed by (41.13) recorded from oven dried mushrooms. The total phenolic (mg GAE/g) content and vitamin C (mg/100g) varied concurrently with same treatments. They were both higher in mushrooms dehydrated with silica gel (174.77) and (175.18) and lower in mushrooms dipped in boiling water for 5 minute (59.49) and (20.17), respectively. These findings demonstrated that *Coprinus cinereus* is a good source of protein and antioxidant and the best post harvest method is sun drying in which, not only the shelf life of the mushroom increases but also they retains most of good qualities including improved scavenging abilities.

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Key words: Antioxidants, *Coprinus cinereus*, proteins, post harvest, scavenging ability.

1.0 Introduction

For many years, nutritional properties and health benefits of mushrooms have been recognized in different parts of the world. Mushroom apart from being delicacy and tasty foods, they are now considered as functional food, being a source of valuable biological substances [1]. They also contain special biochemical compositions, with significant contents of antioxidant compounds, proteins, carbohydrates, lipids, enzymes, minerals, vitamins and water, which attract more attention as functional health promoters [2, 3, 4, 5, 6]. Recently mushrooms have also become an attractive source for the development of drugs, nutraceuticals, antioxidants and generally have been linked to a number of potential health benefits, including protection against heart disease and cancer [5].

Mushroom of the genus *Coprinus* are commonly known as inky cap mushrooms because of their deliquescence nature forming black mass as they mature. The genus is well known for harbouring edible medicinal mushroom distributed almost in all continents of the world. They usually grow on rotting plant materials such as sisal wastes, sugar cane filter mud and some decaying tree logs. *Coprinus* mushroom are widely eaten and well known for their antioxidant properties thus sought to be used in the formulation of nutraceuticals and functional foods [7, 8]. In Tanzania *Coprinus* mushrooms locally in Swahili known as 'uyoga taka mkonge' which means mushroom from sisal waste since they abundantly grow in sisal wastes, are highly consumed by different communities. Recently one of the species *Coprinus cinereus* believed to be indigenous to Tanzania has been successfully domesticated at the Department of Molecular Biology and Biotechnology of the University of Dar es Salaam [9, 10]. Although they have been recently successful domesticated in the country and their antimicrobial activities established [11], farmers and consumers face challenges of their short shelf life due

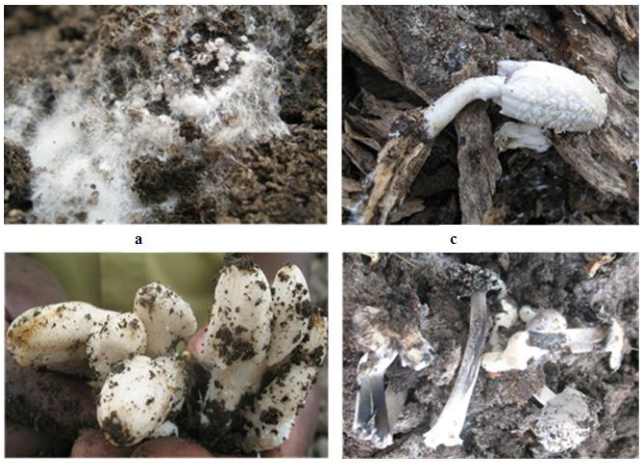


Figure 1: *Coprinus* mushroom growing on sugar cane filter mud (a) mycelia colonizing the mud, (b) young fresh fruit body growing in tufts, (c) Young fresh fruit body growing solitary with white to gray tufts on the basidiocarp (d) Old fruiting bodies deteriorated by deliquescent nature.

to their deliquescent nature.

In an effort to improve their shelf life and maintains high-quality mushrooms and food products, assessment of different post harvest handling methods are indispensable prerequisite. Heat treatment, freezing, sun drying, dehydration and salt drenching are among the common methods applied in different food products to improve their shelf life [4, 12]. However, it is well known that natural nutrients could be significantly lost during different treatments such as the thermal processing due to the fact that most of the bioactive compounds are relatively unstable to heat [13]. Nevertheless, recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to their various chemical changes during heat treatment [14, 15].

This study therefore intended to determine the effect of different post harvest treatment methods on the amount of crude protein and overall antioxidant activities of *Coprinus cinereus* on the way to determine the best post harvest treatment method which while maintain the vital functional compounds in the mushrooms, it helps in improving their shelf life.

2.0 Materials and methods

2.1 *Sample collection and characterisation*

Fresh fruit bodies of *Coprinus cinereus* were collected from sisal processing and sugar factories in Morogoro, Tanga and Kilimanjaro regions during February to May, 2011. Samples were photographed *insitu* (Figure 1) and all macromorphological characters recorded for characterization as described in Tibuhwa et al. [16]. One portion of the collected mushrooms were immediately divided into several portions for various post harvest treatments including immediate dehydration with silica gel in air tight plastic bags, salt drenching, sun drying for 5 consecutive days, immediate freezing, oven dried at 40 ºC, dipping in boiling water for 5 and 1minutes, dipping in boiling 10% salt for 1minute, and a controls of young and immature fresh fruit bodies. Another portion was dried on the an electric mushroom drier (STOCKLI CH-8754, SWITZERLAND) at 50 ºC for 8 hours and preserved for further micromorphological characterization at the Microbiology laboratory of the University of Dar es Salaam, Tanzania. The collected fruiting bodies were identified conventionally based on field guides and already published work of, Arora [17], Härkonen et al. [18], Mshandete and Cuff [9] Tibuhwa et al. [19] and internet facility.

2.2 *Crude extracts preparation and yield determination*

Methanolic extractions were carried out using 1gm of the whole mushroom fruit body weighed using Adventurer TM balance (Ohaus Corp. Pine Brook, NJ, USA) analytical balance at room temperature (29±3ºC). The mushrooms were finely crushed using motor and pestle, and each 1gm of the crushed mushroom material was soaked in 250 ml of methanol as explained in Jaita et al. [20]. It involved constant stirring of the material for 48 hrs then filtered using whatman filter paper (Whatman, GmbH, Dassel, Germany). The filtrates were evaporated to dryness at 40ºC in a rotary evaporator with 90 rpm (Labrota 4001, Heidolph® Essex Scientific Laboratory Supplies Ltd) under reduced pressure. The obtained concentrated extracts were stored in dark at 4ºC until further analysis. The yields of evaporated dried extracts were obtained by gravimetric method. The percentage yield extracts were calculated based on dry weight as:

$$\text{Yield (\%)} = \frac{(W1 \times 100)}{W2}$$

Where W1= weight of extract after methanol evaporation

W2 = Weight of the grinded mushroom powder

2.3 *Determination of antioxidant content*

2.3.1 *Total flavonoid assay*

The total flavonoid was determined with Aluminium chloride according to Jaita et al. [20] using quercetin as standard. One ml of each extract was diluted with 4.3 ml of 80 % aqueous ethanol containing 0.1 ml of 10% Aluminium nitrate and 0.1ml of 1M aqueous Potassium acetate. After 40 minutes incubation at room temperature (29±3ºC) the absorbance was determined calorimetrically at 415 nm using spectrophotometer (Uv-vis model 6305 Jenway UK). Total flavonoid concentration was calculated using quercetin standard calibration curve. Data were expressed as Rutin equivalent/100g of mushroom extracted (mg RE/g).

2.3.2 *Total phenolic assay*

The total phenolic content in each crude methanolic extracts of differently treated mushrooms were determined using the Folin-Ciocalteu colorimetric method [21]. Each 0.1 gm of concentrated extract was diluted with 5 ml of methanol. Two hundred (200) µl of the mushroom extract was then transferred into a test tube and mixed thoroughly with 1ml of Folin-Ciocalteau reagent. After 3 min, 0.8 ml of 7.5% (w/v) Sodium carbonate was added to the mixture. The mixture was agitated for further 30 minutes in the dark and centrifuged at 3300 g for 5 minutes. The absorbance of mushroom extract and prepared blank were measured at 765 nm using spectrophotometer (Uv-vis model 6305 Jenway UK). The total phenolic content in the mushroom extract was expressed as milligram of gallic acid equivalent per 100 g weight of mushroom (mg GAE/g) using the

linear equation obtained from standard gallic acid calibration curve.

2.3.3 *Vitamin C assay*

The vitamin C content was determined titrametrically using 2, 6 Dichloropheno Indophenol methods. Known weight of grounded sample was mixed with 25 ml of 5% metaphosphoric acid solution and shaken for 30 minutes. The mixture was then filtered through Whatman no 42 filter paper (Whatman, GmbH, Dassel, Germany) using sunction pump (Yamato, Handy Aspirator WP--25, Yamato Scientific CO, LTD, Japan). Ten (10) ml was pippeted from the extract in 250 ml conical flask and titrated against 0.025% of 2.6 Dichlorophenol Indophenol reagents. The amount of vitamin C (mg/100g) in each extract was calculated from the equation:

$$\text{mg of ascorbic acid per 100g} = \frac{\text{Ax Ix V1 x 100}}{\text{V2xW}}$$

Whereas A = quantity of ascorbic acid (mg) reacting with 1ml of 2, 6 Indophenol

I = volume of indophenol (ml) required for the completion of extract titration

V1 = Total volume of extract and

W = Weight of the grinded mushroom

2.3.4 *Scavenging ability on* (1, 1-diphenyl-2-picryl-hydrazyl) *(DPPH) radicals*

The qualitative assays of the free radical scavenging ability were performed according to the method of Masuda et al. [22] and Jaita et al. [20]. A series of methanol crude extracts solutions ($1{:}10 - 1{:}10^6$) were prepared. One ml of each of the solution was mixed with 1ml of 0.4 mmol-1 methanolic solution containing a very stable radical, 1:1−diphenyl−2picryhydrazyl (DPPH). The mixture was left in the dark for 30 minutes and the absorbance measured at 515 nm. The percentage of DPPH radical scavenging activity of each extract was determined for the six concentrations ($1{:}10 - 1{:}10^6$) within the range of dose response and was calculated as:

$$\text{DPPH radical scavenging activity} = A_0 - \frac{(A_1 - A_s)}{A_0} * 100$$

Where A_0 = Absorbance of the control solution containing only DPPH

A_1= absorbance in the presence of mushroom extract in DPPH solution and

A_s = the absorbance of the sample extract solution without DPPH

The DPPH radical scavenging activity at each concentration from different post harvest treatments were determined and recorded in Table 1. The total antioxidant necessary to decrease the initial DPPH radical concentration by 50% (LD_{50} value) was determined from plotted graph of scavenging activity against the concentration of extracts in Table 1.

Table1: Scavenging ability of *Coprinus* mushroom treated with different post harvest methods

Treatment	Yield mg/1000ml	10	10^1	10^2	10^3	10^4	10^5	10^6
Oven dried at 40 ºC	1.43	93.47	42.83	39.99	32.53	6.57	6.28	4.71
Sun dried for 5 days	1.23	97.12	35.29	32.23	28.39	26.16	21.24	8.33
Frozen direct	1.72	91.59	58.24	46.65	33.47	20.99	16.12	17.60
Silica gel dehydrated	1.28	98.33	94.18	52.53	41.18	28.24	20.14	18.12
Salt drenched	1.44	97.52	76.71	43.12	40.00	33.88	16.35	5.23
Boiled 5 minutes	1.26	93.52	53.24	48.93	47.82	28.88	19.24	12.12
Boiled 1minutes	1.22	94.41	63.88	49.59	47.29	44.47	36.82	27.55
Boiled-10% salt/minute	1.42	96.53	92.18	58.71	37.94	34.71	32.06	23.11
Fresh- control	1.32	94.12	95.35	48.76	40.94	33.48	25.12	24.88

2.4 *Crude protein determination*

A known weight of each mushroom sample was measured using Adventurer TM balance (Ohaus Corp. Pine Brook, NJ, USA) analytical balance. The weighed samples were then digested using micro Kjeldahl method. After completion of digestion organic nitrogen was determined calorimetrically using Indophenol-blue method using NH_4^+-N as standard. The UV absorbance of the solution was measured using spectrophotometer (Thermo Spectronic Helios β, England) at 660 nm. The total crude protein was obtained by multiplying results (Total Nitrogen) by 6.25 factor according to Allen [23].

2.5 ***Statistical analysis***

All experiments analyses were carried out at least in triplicates to ensure reproducibility and all data were expressed as mean ± S.D. The data for protein, scavenging ability, flavonoid content, phenolic content, vitamin C content from the eight post harvest treatments were subjected to analyses of variance (one-way ANOVA) at the 5% level (significant different at $p<0.05$) using the Statistical Package for Social Sciences (SPSS) Program Version 15.0 [24].

3.0 Results and discussion

3.1 *Characterization of the studied species*

Comparative studies with *Coprinus* species has shown that the present described specimens concurs with the one formerly described by Härkonen et al [18] from Tanzania; it is comparable to *Coprinus cinereus*. The characteristic features of *Coprinus* specimens, collected from the study sites were parallel sided gills which undergoes deliquescence from the base upwards immediately following spore discharge, bluish grey colour of the mature cap, bulbous stipe and long pseudorrhiza which is edible, black to violet - black spore print. These as well as the basidia and basidiospore shape and dimensions were the chief taxonomic criteria of the studied specimens. The identity of the *Coprinus* species specimens in our study concurs with *Coprinus cinereus*, the one formerly described by Härkonen *et al.* [18] from Tanzania. These *Coprinus cinereus* mushrooms were found growing in clusters (small groups) or solitary, on moist alkaline pale grayish coloured sisal waste compost and sugar cane filter mud (Figure

1b&c). However, it would be imperative to conduct higher resolution species identification of this Tanzanian *Coprinus* mushroom using molecular biology techniques.

3.2 *Effect of post harvest treatment on protein, antioxidant and scavenging abilities*

Antioxidant activities of the *Coprinus cinereus* was evaluated in terms of the scavenging ability on DPPH radicals, total flavonoid, total phenolic content, ascorbic acid (Vitamin C) and crude protein according to Adefegha and Oboh [25]. The effects of post harvest handling methods namely oven drying at 40ºC for 24 hours, sun drying for 5 days, freezing, dehydrating with silica gel, drenching (keeping) in saturated salt, dipping in boiling 10% salt for one minute, dipping in boiling water for one minute and 5 minutes were investigated. Young and immature (fresh fruit bodies) *Coprinus* mushrooms were also included as control without being treated. All the samples under different post harvest treatments revealed antioxidant properties and scavenging abilities but with marked variations. For example, the scavenging abilities were in the order sun dried > fresh control > dipping in boiling water for one minute > dehydrating with silica gel > salt drenched > oven drying at 40ºC for 24 hours > freezing > dipping in boiling 10% salt for one minute. In the present study, a strong correlation was found between the values of scavenging ability and total phenolic content. This suggested that the reducing activity of phenolic compounds is an important factor dictating the free radical scavenging capacity of these compounds. This finding is in line with other studies, which also noted that the scavenging ability in mushrooms is mainly associated with the total phenolic content they contain, the higher the phenolic content the best the scavenging power [6, 26, 27]. *Coprinus cinereus* mushrooms seem to be rich source of antioxidants, protein and have high antiradical potentials (Table 1). The result findings show that the best post harvest method was sun drying, in fact it showed high free radical scavenging ability (LD_{50} = 0.119), which is of higher ability than

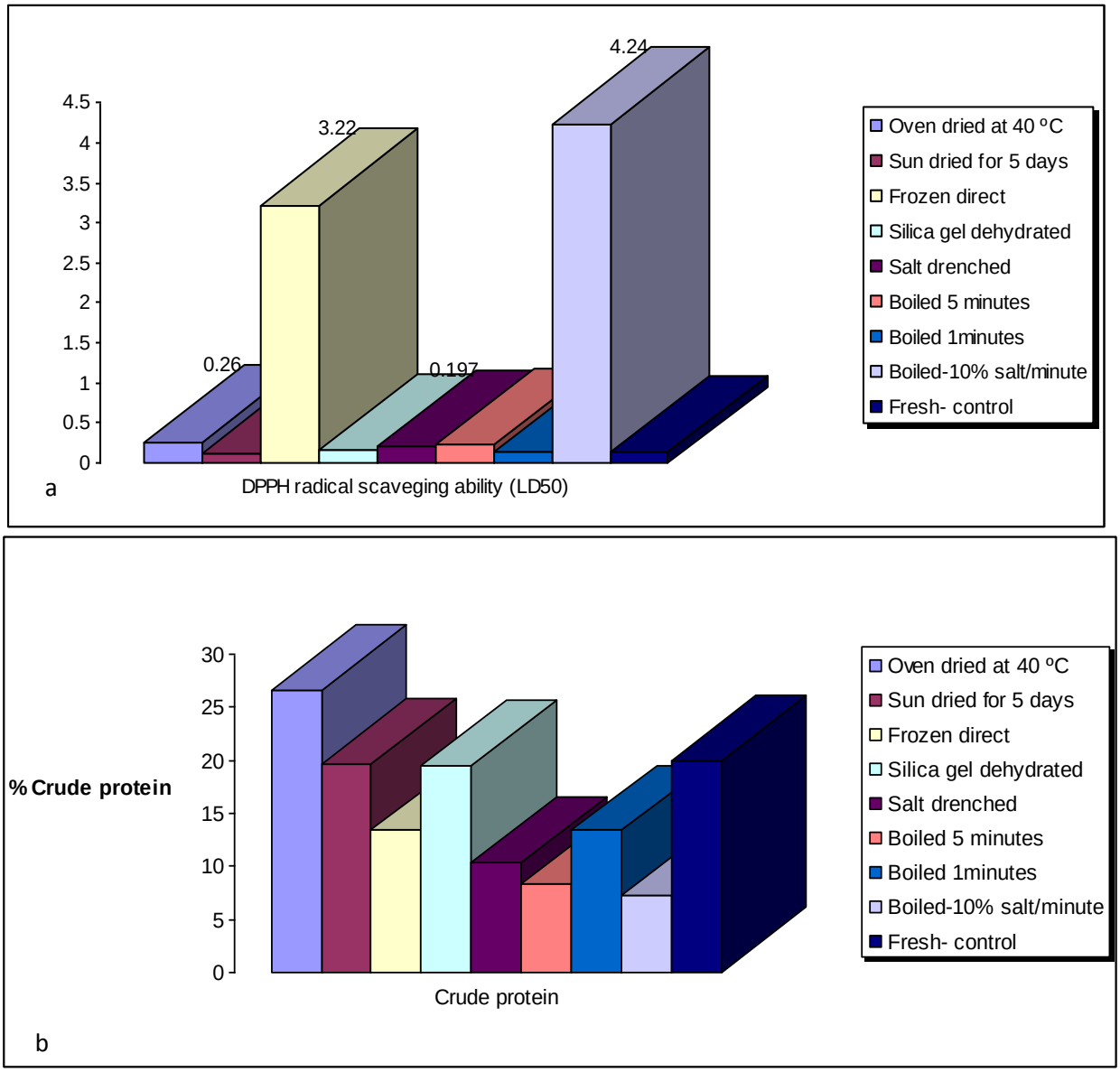
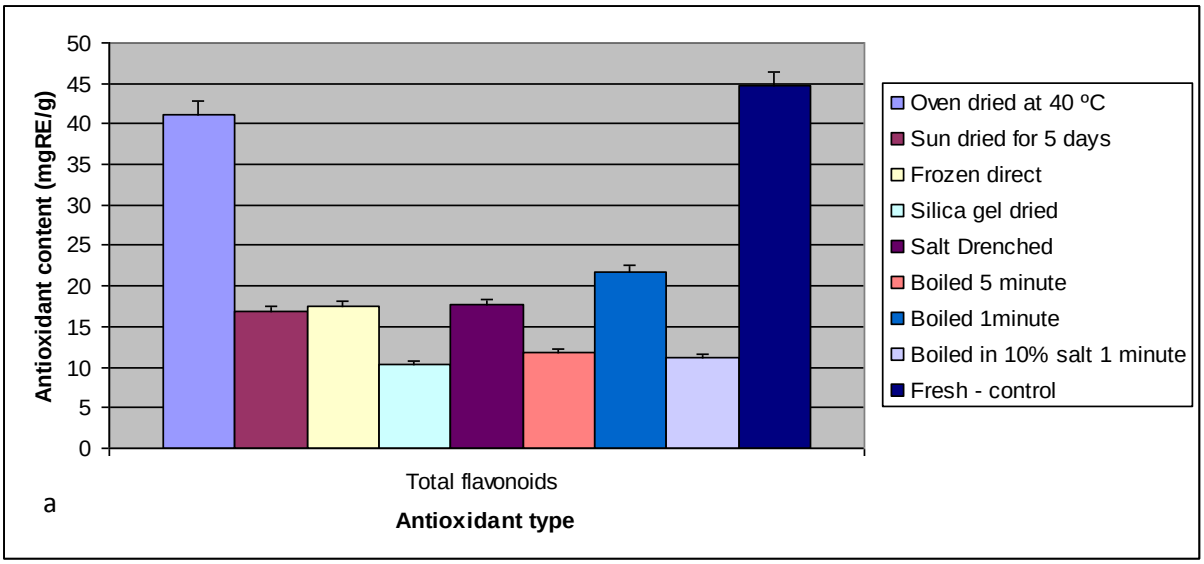
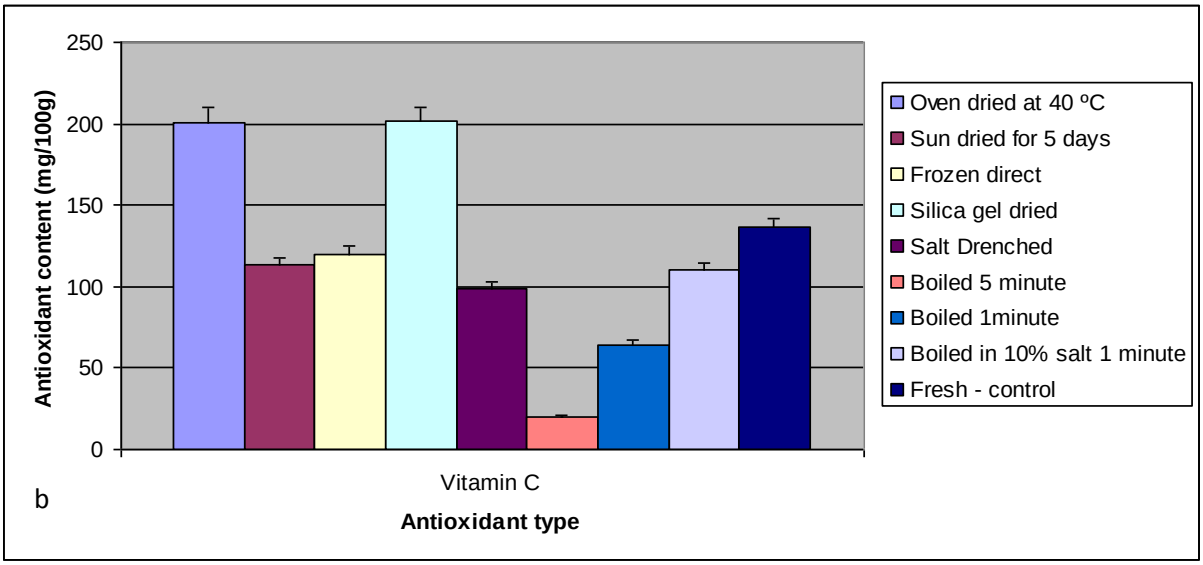


Figure 2: (a) DPPH radical scavenging ability and (b) crude protein content in different Post harvest treated mushroom





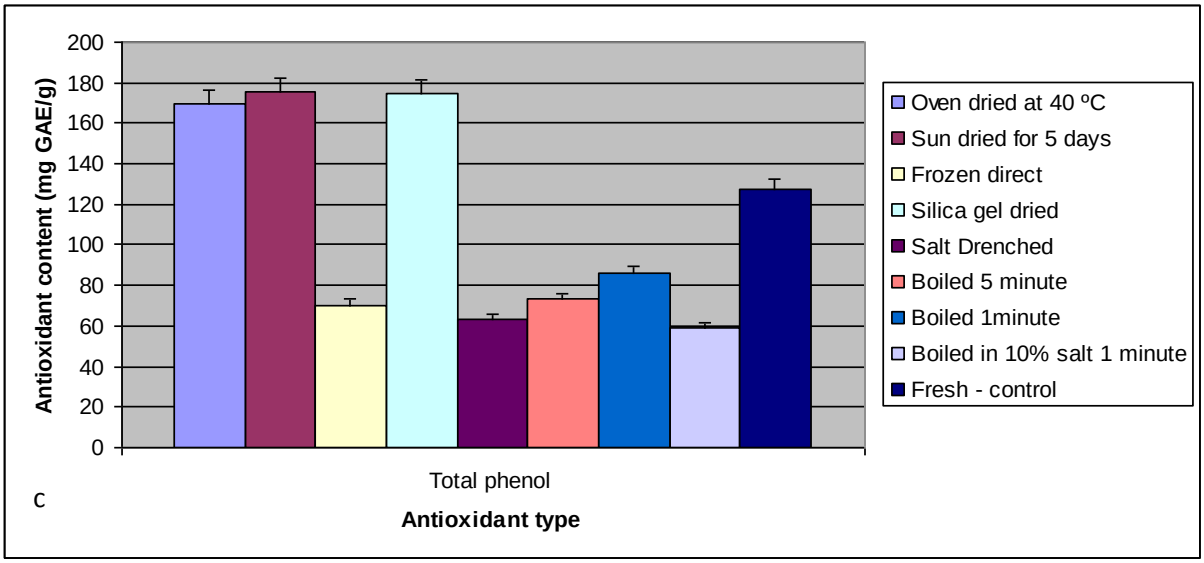


Figure 3: Content of crude protein and antioxidants of different post harvest treatments. Error bars indicate standard error of the mean of the replicates

(LD_{50} = 0.133) of the untreated fresh control (Figure 2). In this study, it was also found that, sun drying process enhanced the scavenging ability of the mushroom by 11% (compared to young and immature fresh fruit bodies (control), while treatment by temperature such as dipping the mushrooms in boiling water with or without salt, decreased the scavenging ability, antioxidant content, and protein content. For example, both least values of scavenging ability (LD_{50} =4.24) and percentage crude protein (7.31%) were from samples treated by dipping mushroom in boiling 10% salt water for one minute. Also for total phenolic content (mg GAE/g) and vitamin C (mg/100g) both least values were lower in boiled sample (59.493) and (20.17), respectively (Figure 2, 3). This result is contrary to the observation by other researches who observed increased polyphenolic contents and antioxidant activities with thermal treatment in *Lentinula edodes* mushroom [12] and other food products [13]. The total phenol content of the mushrooms under different post harvest treatment method ranged from 175.188 mg/100 g (sun dried for 5 days) to 59.493 mg/100 g (boiled in 10% salt for 1 minute). Compared with the young and immature fresh fruit bodies which were used as control, the highest level of increase in total phenols occurred in sun dried for 5 days, whereby sun drying caused 27% gain, while boiled in 10% salt 1 minute had the loss of (87%) in total of phenol content. This result indicates that most of the chemical structures of phenol compounds trapped in fibre of the mushrooms are affected differently with different post harvest treatment method. This finding second the results of Rice-Evans [28] who found out that the antiradical activity of phenols is principally based on the structural relationship between different parts of their chemical structure. Since phenolic compounds are well known as secondary metabolites commonly found in mushrooms and reported to have vital biological functions including antioxidant activity [27, 29]; this study therefore, recommended to chose post harvest treatment methods which have no direct effect to the chemical structure of the phenolic compounds, in order to maintain their antioxidant potential.

Conclusion

The extracts of *Coprinus cinereus* indicated that is effective good scavenger of free radicals and contain antioxidants. In view of the present results it could be concluded that *Coprinus cinereus* is a rich source of protein and antioxidants, however, different preservation treatment can increase or decrease protein content and quantity and quality of antioxidant studied. Sun drying can be the best method for post harvest preserving the taxa with the advantage of increasing its scavenging ability while improving their shelf life. Therefore *Coprinus cinereus* can serve as a dietary supplement for proteins, vitamin C and easily accessible source of natural antioxidants. However, intensive and extensive research needed to exploit their valuable therapeutic potential.

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