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Extensive Study of Antioxidant Activity in *Agaricus Bisporus, Calocybe Indica* and *Pleurotus Ostreatus* Under Varying Cooking Conditions

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I. Introduction

Oxidation is the key factor for the living organisms in the production of energy for biological processes. Although oxidation is necessary, excess formation of oxygen - derived free radicals initiates many disorders such as tumor development, autoimmune diseases and also in degenerative processes associated with ageing [1]. These highly reactive oxygen derived free radicals which are capable of oxidizing biomolecules leading to cell death and tissue damage are produced by chemical and metabolic processes of the human body [2]. Almost all organisms are well protected against free radicals induced damage by oxidative enzymes such as super oxide dismutase (SOD), catalase (CAT), antioxidants such as α- tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione [3].The antioxidants present in the plant foods serves as a protection by scavenging free radicals. In addition to naturally occurring antioxidants the most commonly used synthetic antioxidants are butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and tert-butylated hydroxyquione (TBHQ). [4]. Some synthetic antioxidants have been reported to be carcinogenic, hence research on the potential applications of natural antioxidants, have received much attention [5-8].

Mushrooms are readily available source for water soluble vitamins. Edible mushrooms in cooked or other processed forms are rich in nutrients and antioxidants [9] and are suitable for diabetic and heart patients. Some recently isolated and identified compounds in mushrooms show significant medical properties, such as immuno-modulatory, cardiovascular, liver protective, anti-inflammatory and anti-diabetic [10-13].

Many plant products are studied for antioxidant property, but, till date, the effects of cooking and comparison of cooking methods on antioxidant capacity of foods have not been well studied. [14].Though there are studies on antioxidant activity of mushrooms, only little information is available about the effect of cooking on antioxidant activity of the mushrooms. In this study, we explored the effects of three cooking methods (boiling, steaming and microwaving) on antioxidant capacity of edible mushroom species *Agaricus bisporus, Calocybe indica* and *Pleurotus ostreatus* .

II. Materials and Methods

a) Chemicals

All chemicals used were of analytical grade; 1,1- Diphenyl – 2 – picryl –hydrazil (DPPH), ferrous chloride, 3- (2-pyridyl) -5,6-bis (4- phenyl – sulfonic acid- 1,2,4-triazine) (Ferrozine), trichloroacetic acid (TCA), potassium ferric cyanide, ferric chloride, cerium

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sulphate and ethanol were obtained from Sigma chemicals, USA and Himedia chemicals, Mumbai, India.

b) Standards

The three standards used in this study are reported to remove the oxygen free radicals and acts as antioxidants. The standards were obtained from Sigma chemicals, USA.

a. Quercetin – Flavonoid – $C_{15}H_{10}O_7$
b. Rutin - glycoside between the quercetin and the disaccharide rutinose - $C_{27}H_{30}O_{16}$
c. Ascorbic acid - Form of Vitamin C - $C_6H_8O_6$

c) Plant Material and Extraction

Three mushroom species *A. bisporus, C. indica* and *P. ostreatus* were commercial samples. Fresh mushrooms from each species were divided into small pieces; 20g was weighed and grounded by using homogenizer, then mixed with aqueous solvent, water in room temperature at 150rpm for overnight. The extract was filtered over Whatman No.1 filter paper and the filtrate was collected, solvent was removed by evaporating at 50°C to obtain the dry extract. The extracts were placed in a plastic bottle and then stored at 4°C to prevent oxidative damage until analysis of the extracts.

d) Cooking Methods

The antioxidant activity of cooked mushrooms is analyzed by adapting three common cooking methods *viz.,* microwaving, steaming and boiling.The mushrooms were washed under running tap water and remaining water is blotted before weighing.

i. *Microwaving*

Mushrooms of 50g and 200ml of distilled water were added to a glass beaker and micro waved for 5 minutes. The mushrooms were then cooled for few minutes to room temperature and homogenized then the extract was prepared as stated above.

ii. *Steaming*

50g of mushroom was placed over 95°C water in a closed water bath for 5 minutes, after which the mushrooms were removed, cooled and processed as above.

iii. *Boiling*

200ml of boiled distilled water were added to a 50g portion of mushroom and placed in water maintained at 95°C for 5 minutes. After cooking the mushrooms were cooled, homogenized and extracted as above.

e) Determination of total Antioxidant Activity

The total antioxidant activities of the extracts were determined by the cerium (IV) sulphate method [15]. Cerium (IV) sulphate solution of 2mM concentration was added to extracts and standards of varying concentrations (100-1000μg/ml). Each concentration is done in triplicates. The solution is mixed well and incubated for 30minutes at room temperature. Absorbance of each solution was measured at 320nm using UV/ VIS spectrophotometer (Beckman DU-530).

f) Determination of Reducing Power

The reducing power of the aqueous extracts was determined by ferricyanide method [16]. Varying concentrations of standards and aqueous extracts (100-1000μg/ml) were mixed with phosphate buffer (0.2M, pH 6.6) and 1% potassium ferricyanide. The reaction was incubated at 50°C for 20 minutes and rapidly cooled followed by addition of 10% trichloroaceticacid. The contents are centrifuged at 1000g for 10 minutes. The supernatant obtained was mixed with 0.1% ferric chloride and allowed to stand for 10 minutes at room temperature. The absorbance was measured at 700nm using UV/ VIS spectrophotometer (Beckman DU-530).

g) DPPH radical scavenging assay

The free radical scavenging ability of the extracts was determined by using $DPPH^+$ [17]. DPPH, (1, 1-Diphenyl-2-picryl-hydrazil) radical solution of 1mM was added to varying concentration (100-1000μg/ml) of test solution and standards. The reaction mixture was mixed and incubated at room temperature for 30 minutes. The absorbance was recorded at 517 nm using UV/ VIS spectrophotometer (Beckman DU-530). $DPPH^+$ scavenging activity was determined as:

$DPPH^+$ scavenging activity (%) = [1-(test sample absorbance/ blank sample absorbance] × 100.

h) Ferrous ion Chelating Ability

The ferrous ion chelating ability [18] was used to determine the metal chelation ability of the mushroom extract. The varying concentrations (100 – 1000μg/ml) of extracts and standards were mixed with 2mM $FeCl_2$ and 5mM ferrozine solutions. The reaction was incubated for 10 minutes at room temperature. The absorbance at 562nm of the resulting solutions was measured and recorded using UV/ VIS spectrophotometer (Beckman DU-530). The ion chelating ability is calculated by using the formulae:

Ferrous ion chelating ability (%) = [1-(test sample absorbance/ blank sample absorbance] × 100

i) Statistical Analysis

The experimental data were evaluated and graphs were plotted by using Microsoft Excel. The statistical analysis such as one way ANOVA was performed using Graphpad prism 5.0 software

III. Results and Discussion

a) Determination of Cerium (IV) sulphate antioxidant capacity

The Cerium (IV) sulphate assay was based on the oxidation of antioxidant by Ce (IV) ions. The absorbance indicates the concentration of unreacted Ce (IV) ions. The antioxidant capacity of samples increases

with increasing concentration and this was observed from the decrease in absorbance.

The total antioxidant activity of *P.ostreatus* was found to be higher than *A.bisporus* and *C.indica*. The antioxidant activity of cooked mushrooms was observed to be higher than the raw ones [Figure 1a-e]. Among the cooking processes performed the antioxidant activity is higher in the steam cooked mushrooms. The antioxidant activity of all three steam cooked mushrooms were plotted separately for detailed explanation. These differences were statistically significant at 5% level ($P<0.05$) using one way ANOVA.

b) Determination of Reducing Power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [19]. Fe^{3+} - Fe^{2+} transformation was investigated in the presence of sample for the measurements of the reductive ability. In this method, the antioxidants reduced the oxidant probe Fe^{3+} to Fe^{2+}. This ion then conjugated with the ferricyanide ion to form a Prussian blue coloured product, which was spectrophotometrically measured at 700nm. An increase in the absorbance was due to an increase in the extract concentration, which indicates a significant reducing power [Figure 2a-e]. The reducing power of the fresh mushrooms were in the order of *P.ostreatus*> *A.bisporus* >*C.indica*. The reducing power of the cooked ones was higher than the raw mushrooms. The reducing power was also higher in the steam cooked mushrooms than the other ones which are shown in Figure 2. These differences were statistically significant at 5% level ($P<0.05$) in one way ANOVA.

c) DPPH Radical Scavenging Assay

The DPPH reacts with methanol or absolute ethanol to give a purple colour DPPH radical ($DPPH^{+}$). The presence of antioxidants in the sample will scavenge the formed DPPH radical and there by a decreasing the formed colour. Scavenging effects of extracts from mushroom species on DPPH radicals increased with increasing concentrations. [20, 21]

The DPPH radical scavenging ability for fresh *P.ostreatus*, *A.bisporus* and *C.indica* were 44.7, 42.7 and 40.3% respectively at the concentration of 1000μg/ml. The scavenging ability for steam cooked *P.ostreatus*, *A.bisporus* and *C.indica* were 94.4, 91.8 and 89.3% respectively. The free radical scavenging ability for methanolic extracts of *A.bisporus* and *P.ostreatus* were reported to be 77.5 and 81.3% at the concentration of 180 μg/ml [1]. The scavenging ability was found to be lower for aqueous extract than methanol extract yet methanol was not preferred in this study because of its toxic nature. The radical scavenging ability for three mushrooms under varying cooking conditions along with fresh ones and standards were depicted in [Figure 3a-e].

The one way ANOVA analysis shows that fresh and cooked mushrooms were found to be significantly different.

d) Ferrous ion chelating ability

In this assay the mushroom extracts and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator.

Ferrous ion chelating ability for *P.ostreatus*, *A.bisporus* and *C.indica* were 93.0, 91.3 and 89.6% respectively at the concentration of 1000μg/ml. The metal chelating ability for steam *P.ostreatus*, *A.bisporus* and *C.indica* were 91.3, 89.8 and 87.2% respectively. The metal chelating ability for methanol extracts of *A.bisporus* and *P.ostreatus* were 58.5 and 62.5% at the concentration of 100μg/ml [1].

These differences were statistically measured at 5% level ($P<0.05$) using one way ANOVA. The difference is not statistically significant. This shows that ion chelating ability though reduced to certain extent in cooked mushrooms than the raw ones it is not significant reduction. The ferrous ion chelating ability for fresh mushrooms along with the cooked ones were shown in [Figure 4a-e].

IV. Conclusions

Based on the results of this study, it is clearly indicated that the aqueous extracts of fresh and cooked mushroom species *A.bisporus, C.indica* and *P.ostreatus* has significant antioxidant activity against various antioxidant systems *in vitro.* The chelating ability of *P.ostreatus* was found to be similar to the potent antioxidant standards, rutin, quercetin and vitamin C. The results presented here clearly demonstrate that process of cooking can make the antioxidant capacity of cooked food quite different from that of uncooked ones. This is most probably caused by a variety of effects, including destruction, release and transformation of food components. In this study, steaming was shown to be best method to retain and/or enhance the antioxidant capacity, followed by boiling and then by microwaving. To conclude, steaming is the preferred method to enhance the potential to obtain antioxidants from mushrooms. Hence these mushroom species can be used as an easily accessible source of natural antioxidants in fresh and cooked forms and also as a possible food supplement or in pharmaceutical industry.

References Références Referencias

1. Elmastasa, M., Isildaka, O., Turkekulb, I., & Temura, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms.

Journal of Food Composition and Analysis, 20, 337–345.
2. Halliwell, B., & Gutteridge, J. M. C. (2003).Free radicals in biology and medicine. Oxford, UK: Oxford University Press).
3. Niki, E., Shimaski, H., & Mino, M. (1994).Antioxidantism – free radical and biological defence Tokyo: Gakkai Syuppan Center.
4. Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. Food Chemistry, 81, 249–255.
5. Aruoma OI, Spencer JP, Rossi R, Aeschbach R, Khan A, Mahmood N, Munoz A, Murcia A, Butler J, Halliwell B.(1996). An evaluation of the antioxidant and antiviral action of extracts of rosemary and Provençal herbs. Food Chem Toxicol, 34(5):449-56.
6. Gu, L., &Weng, X. (2001). Antioxidant activity and components of Salvia plebeia R.Br.-a Chinese herb. Food Chemistry, 73, 299–305.
7. Lim, K., C. Hu and D. D. Kitts. (2001). Antioxidant activity of a Rhus verniciflua Stokes ethanol extract. Food Chem. Toxicol.39:229-237.
8. Madsen, H.L., B.R. Nielsen, G. Bertelsen and L.H. Skibsted, (1996). Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. Food Chem., 57: 331-337.
9. Breene WM (1990). Nutritional and medicinal value of speciality mushrooms. J. Food Protect., 53: 883-894.
10. Gunde-Cimmerman N (1999). Medicinal value of the genus Pleurotus (fr). P Karst (Agaricales s.l. Basidiomycetes). Int. J. Med. Mush., 1: 69-80.
11. Ooi V.E.C., Liu F. (1999): A review of pharmacological activities of mushroom polysaccharides. International Journal of Medicinal Mushrooms, 1: 195–206.
12. Ooi V. E. C., (2000). Medicinally important fungi. Science and cultivation of edible fungi,41–51. Rotterdam: Balkema.
13. Wasser, S.P. and A.L. Weis. (1999a). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. International journal of medicinal mushrooms 1(1): 31-62.
14. Wachtel-Galor Sissi, Wong Ka Wing and Benzie Iris FF, (2008)The effect of cooking on *Brassica* vegetables, *Food Chem*,110 (3), 706-710
15. Res et Apak, Birsen Demirata, Dilek Ozyurt, (2007). Determination of total antioxidant capacity by a new spectrophotometric method based on Ce(IV) reducing capacity measurement, Talanta, 72:1157-1165M.Oyaizu, *Journal of Nutrition*, 44, 307- 315 (1986).
16. Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.
17. Decker, E.A and Welch,B,(1990), Role of ferritin as a lipid oxidation catalyst in muscle food, Journal of Agricultural and Food Chemistry,38, 3,674–677.
18. Meir,S.,Kanner, J., Akiri,B and Hadas,S.P., (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves, Journal of Agricultural and Food Chemistry,43, 1813–1817.
19. Duh, P.D., Tu Y.Y., and Yen, G.C., (1999). Antioxidant Activity of the Aqueous Extract of Harnjyur (Chrysanthemum morifolium Ramat), Lebensmittel-Wissenschaft and Technologie, 32, 269-277.
20. Chang, L.W., Yen, W.J., Huang, S.C., and Duh, P.D., (2002).Antioxidant activity of sesame coat, Food Chemistry, 78, 3,347–354.
21. Yamaguchi, F., Ariga, T., Yoshimira ,Y., & Nakazawa, H. (2000). Antioxidant and anti-glycation of carcinol from Garcinia indica fruit rind. Journal of Agricultural and Food Chemistry, 48,180–185.

Contribution by Authors

K.B – Performed experiment, data analysis and paper work.
R.P – Acted as study director and provided trouble shooting support.
S.T – Co-ordinated and designed the study.

Figures Legends

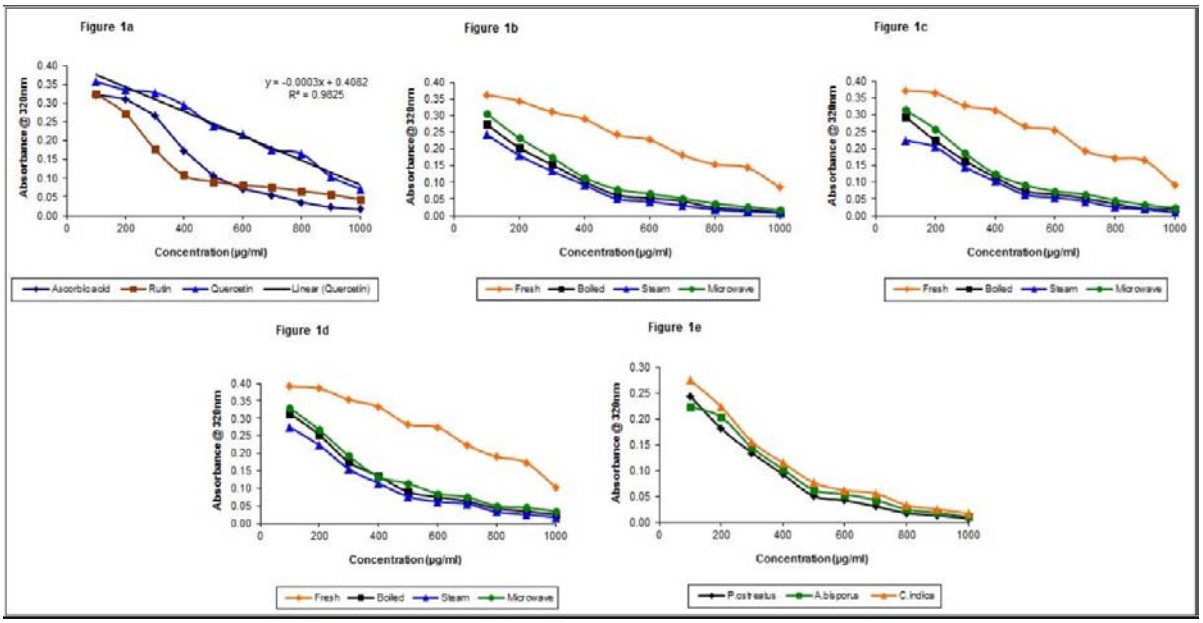

Figure 1a : Standard graph for total antioxidant activity.
b : Total antioxidant activity of P.ostreatus in cooking conditions.
c : Total antioxidant activity of A.bisporus in cooking conditions.
d : Total antioxidant activity of C.indica in cooking conditions.
e : Total antioxidant activity of steam cooked mushrooms.

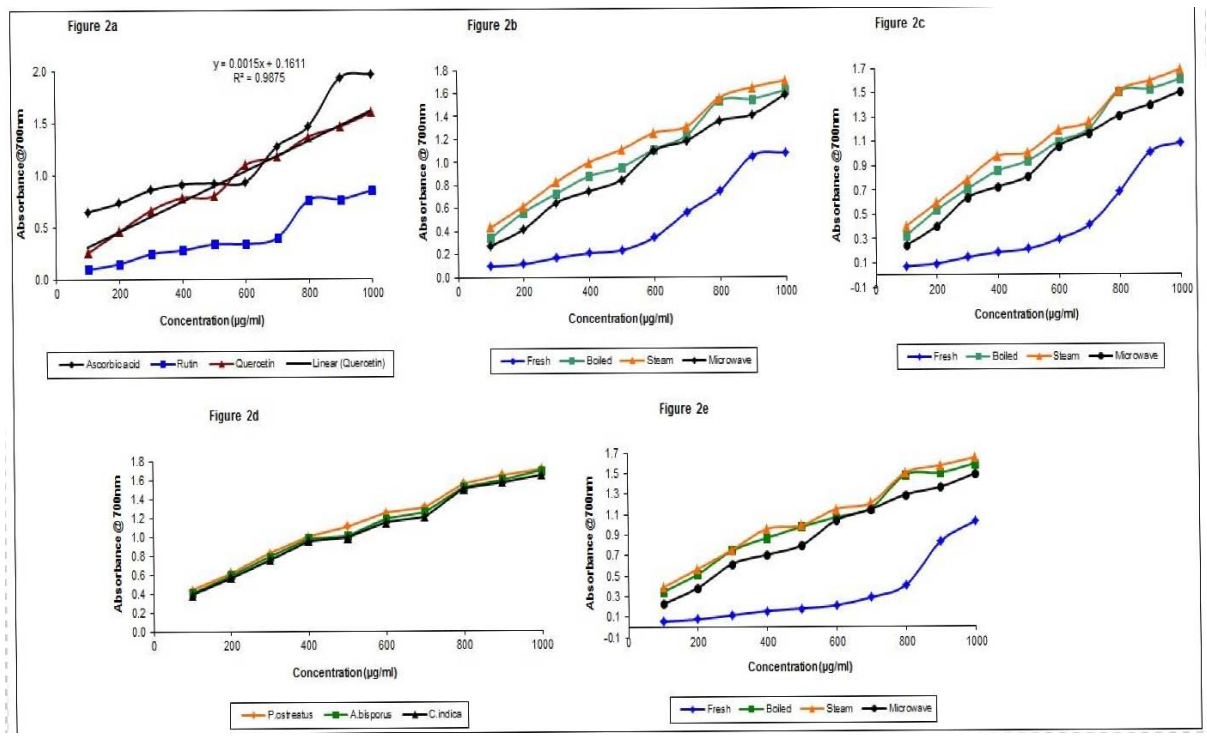

Figure 2a : Standard graph for determination of reducing power.
b : Reducing power of P.ostreatus in cooking conditions.
c : Reducing power of A.bisporus in cooking conditions.
d : Reducing power of C.indica in cooking conditions.
e : Reducing power of steam cooked mushrooms.

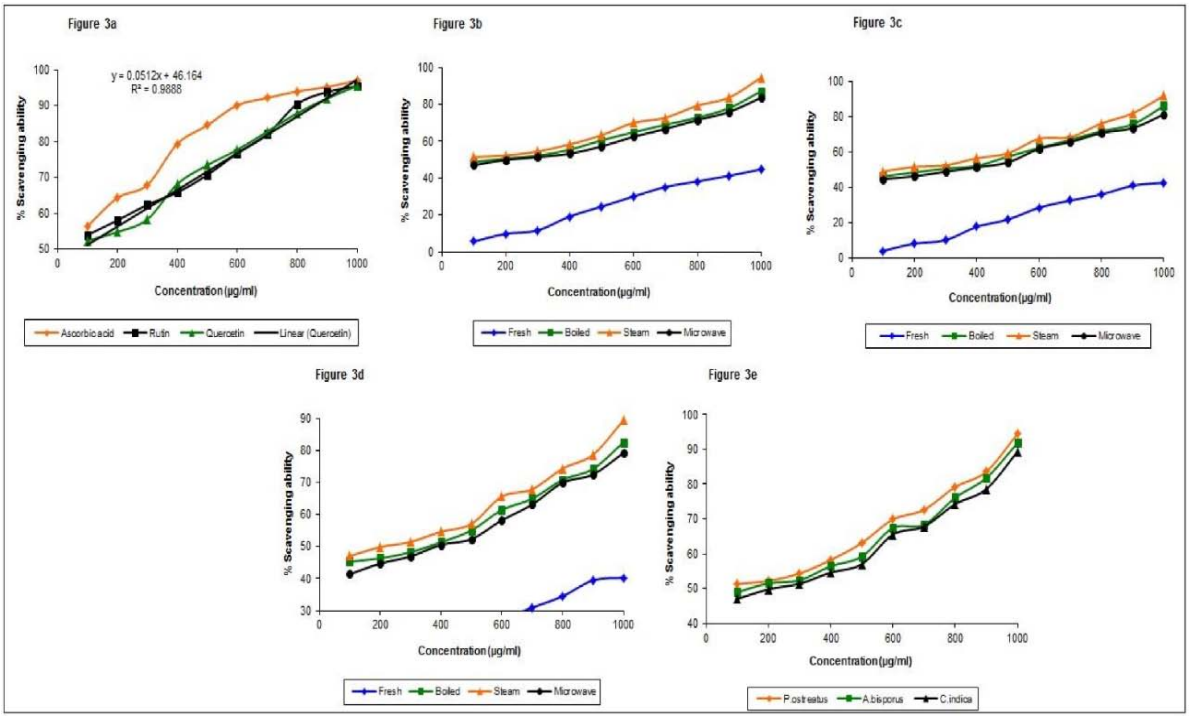

Figure 3 a : Standard graph for DPPH radical scavenging ability.
b : Radical scavenging ability of P.ostreatus in cooking conditions.
c : Radical scavenging ability of A.bisporus in cooking conditions.
d : Radical scavenging ability of C.indica in cooking conditions.
e : Radical scavenging ability of steam cooked mushrooms.

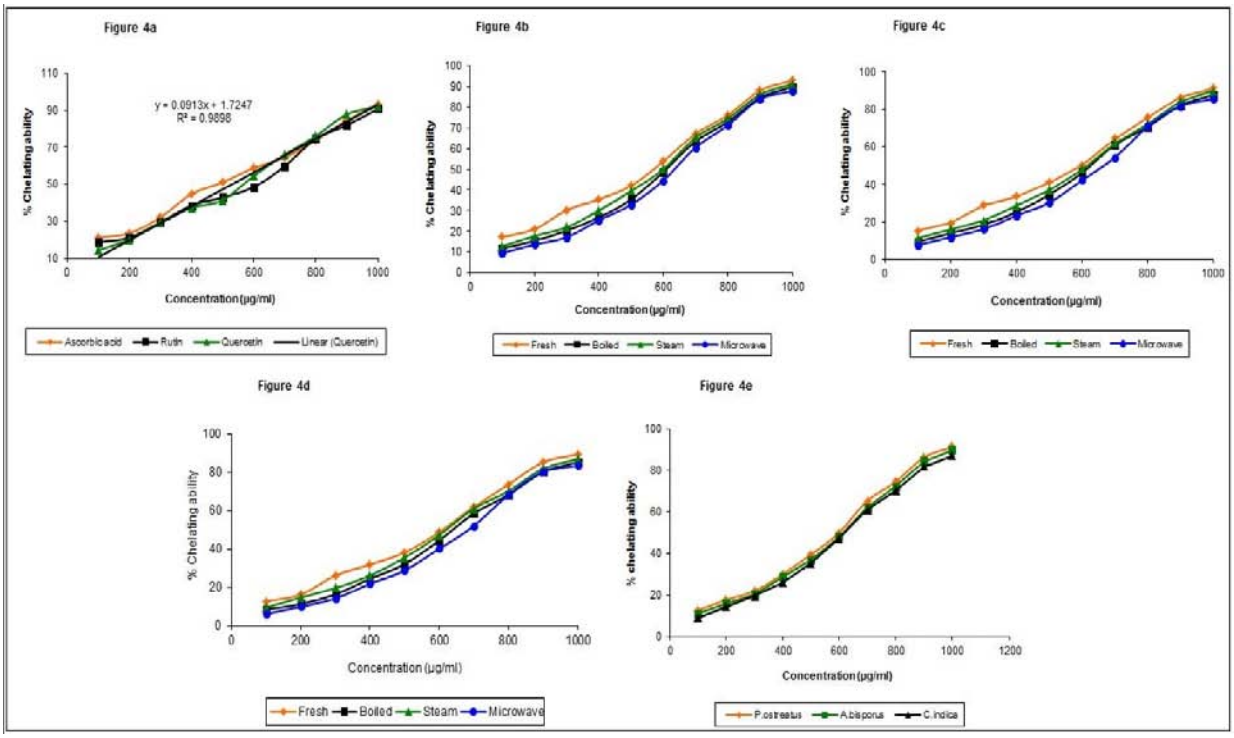

Figure 4 a : Standard graph for ion chelating ability.
b : Chelating ability of P.ostreatus in cooking conditions.
c : Chelating ability of A.bisporus in cooking conditions.
d : Chelating ability of C.indica in cooking conditions.
e : Chelating ability of steam cooked mushrooms.