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Antioxidant Activities and Mineral Composition of Oyster Mushroom (*Pleurotus sajor-caju*) as Influenced by Different Drying Methods

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The aim of the present investigation was to determine the antioxidant activities and mineral composition of oyster mushrooms (*Pleurotus sajor-caju*) as influences by different drying methods. Different dried oyster mushroom samples had 27.3-31.4 mg GAE/g dry wt. of phenolics, 2.19-53.74 % of radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 16.36-94.88 % of radical scavenging activity on 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 61.77-265.08 µM $FeSO_4$ equivalent of ferric reducing antioxidant power (FRAP), 144.03-372.05 µM Trolox equivalent of total antioxidant activity, 0.07-0.30 of reducing power and 23.13-78.12 % of metal chelating activity. Higher amount of nitrogen (4.84 %), potassium (5.07 %), zinc (0.093 mg/g), copper (0.010 mg/g), iron (0.168 mg/g) and manganese content (0.035 mg/g) was found in solar dried oyster mushroom samples. Principal component analysis revealed that radical scavenging activity on DPPH and ABTS are having positive correlation with solar dried *P. sajor-caju*. We found that solar dried oyster mushrooms are rich in different antioxidant activities and minerals composition.

Keywords: Oyster mushroom, Drying method, Antioxidant activities, Mineral composition, Principal component analysis.

INTRODUCTION

Mushrooms have various medicinal and nutritional properties and have used as food purposes since decades [1]. Concentrated extracts of various dried mushrooms are used as medicines and dietary supplements [2]. Mushrooms are also a rich source of protein and mineral especially in vegetarian communities. In recent days, mushrooms have become an attractive functional food mainly because of their nutritional composition [3] and the antioxidant capacity of mushrooms to scavenge free radicals, which are responsible for oxidative damage of human cells. The antioxidants present in mushrooms are of great interest as protective agents to help the human body reduces oxidative damage.

Oyster mushroom is a novel source of easily digestible form of protein than many legume and vegetable [4]. *Pleurotus* spp are also containing high quantities of carbohydrates, micronutrients and vitamins B complex, as well as low fat content [5]. However, mushroom are extremely perishable and the shelf life of most of the mushrooms is only about 24 h at ambient conditions and 7-10 days under controlled conditions because of its high moisture content and rich nutrients that spoil easily and quickly. The various physiological and morphological changes that occur after harvest make these mushrooms unacceptable for marketing hence consumption [6]. The *Pleurotus sajor-caju* is a delicate, sensitive mushroom, so this starts deteriorating immediately within 1 day after the harvest [5]. So its preservation into more value added products is of great importance. To extend the availability and shelf life of oyster mushrooms, processing methods such as sun drying, oven drying, solar drying and atmospheric drying (vacuum and freeze drying) are recommended [6,7]. The dehydrated product offers, increased shelf life and pleasant flavour and also increase the potential for storage and transport of the product [8]. In several reports the nutritional aspects of cultivated and wild edible mushroom have been studied [9,10]. Different drying methods are believed to impact on chemical composition, physico-chemical properties and antioxidant activities of the mushrooms [11,12]. Keeping above in mind, the present investigation was undertaken to study the effect of various drying methods on antioxidant properties and mineral composition of oyster mushroom.

EXPERIMENTAL

Sample preparation: Oyster (*Pleurotus sajor caju*) mushroom cultivated on wheat straw were harvested, washed thoroughly and cut into small pieces. Sliced pieces of mushrooms were subjected to drying treatments. 300 g sample was taken to give each treatment. Each sample was dried from initial moisture content from 91 % on fresh weight basis to the final

moisture content, 10 % on dry weight basis following the standard methodology [13,14]. The dried mushrooms were stored in a vaccum desiccator in a cool place until further analysis.

Extraction of dried mushroom samples: Each mushroom sample (1.5 g) was extracted by stirring with 20 mL methanol and double distilled water separately at 25 °C, 150 rpm for 12 h and filtered through Whatman filter paper No. 1. The extraction was repeated again. The extracts were mixed, filtrated and diluted upto 50 mL with methanol and double distilled water separately. The extract solutions were stored in amber bottles at 4 °C served as the working solution (30 mg/mL) for determination of total phenolics and antioxidant activity.

Determination of total polyphenol: Total polyphenol in the mushroom samples was estimated by a spectrophotometric assay, as described by Singleton and Rossi with minor modifications [15]. Briefly, 1 mL of mushroom extract (10 mg/mL) was taken and into it, 1 mL of Folin and Ciocalteu's phenol reagent was added. After 3 min, 1 mL of 20 % Na_2CO_3 solution was added to the reaction and the final volume of the reaction was adjusted to 10 mL with distilled water. The reaction tubes was kept in dark for 90 min and then the absorbance was taken by Thermo scientific chemito spectra scan UV 2600 spectrophotometer at 725 nm. Gallic acid was used to calculate the standard curve (1-80 µg/mL). The results were mean values ± standard deviations and expressed as mg of gallic acid equivalent (GAE)/g of extract.

Determination of radical scavenging activity on DPPH: Radical scavenging activity (RSA) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by spectrophotometric method as described by Brand-Williams *et al.* [16]. The stock solution containing 24 mg of DPPH in 100 mL of methanol was prepared and stored at -20 °C and the working solution was prepared by mixing 10 mL stock solution with 45 mL methanol to get an absorbance of 1.17 ± 0.02 units at 515 nm. Mushroom extract (150 µL) of various concentrations (5-25 mg/mL) were allowed to react with 2850 µL of DPPH working solution for 24 h in dark and afterwards absorbance was taken at 515 nm. Butylated hydroxy toluene was used as a reference and radical scavenging activity was calculated as a percentage of DPPH free radical:

$$\text{RSA on DPPH (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where, A_{sample} is the absorbance of the sample solution and $A_{control}$ is the absorbance of the DPPH solution without sample. The extract concentration providing 50 % inhibition (EC_{50}) was calculated for scavenging activity comparison.

Determination of radical scavenging activity on ABTS: The radical scavenging activity (RSA) on ABTS was determined by the method of Arnao *et al.* [17] with minor modification. Stock solutions; 7.0 mM ABTS and 2.3 mM ammonium persulfate, were prepared. The working solution was prepared by mixing both the stock solutions in equal quantities and keep the working solution for 12 h in dark then working solution was diluted four times with methanol to get an absorbance of 0.9 ± 0.02 units at 745 nm. 200 µL of sample extract of different concentrations (5-25 mg/mL) were allowed to react with 2 mL of the freshly prepared ABTS solution for 0.5 h in dark condition and read the samples in spectrophotometer at 745 nm. Butylated hydroxy toluene was used as a reference and the percentage inhibition was calculated by the equation:

$$\text{RSA on ABTS (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where, A_{sample} is the absorbance of the sample solution and $A_{control}$ is the absorbance of the DPPH solution without sample. The extract concentration providing 50 % inhibition (EC_{50}) was calculated for scavenging activity comparison.

Determination of ferric reducing antioxidant power (FRAP): The FRAP assay was done according to the standard methodology with some modifications [18]. The stock solutions 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$; pH 3.6), 10 mM 2,4,6-tripyridyl-*s*-triazine solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution were prepared. The fresh working FRAP solution was prepared by mixing 25 mL acetate buffer, 2.5 mL 2,4,6-tripyridyl-*s*-triazine solution and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and warmed at 37 °C before use. Mushroom extracts (150 µL) of different concentrations (5-25 mg/mL) were allowed to react with 2850 µL of the FRAP solution for 30 min in dark condition and absorbance of the coloured product were taken at 593 nm. A standard curve was prepared by addition of ferrous sulfate heptahydrate to the FRAP reagent. The results were expressed in µM equivalent to $FeSO_4 \cdot 7H_2O$ as FRAP value.

Determination of total antioxidant activity: The total antioxidant activity (TA) of mushroom extracts was estimated using the phosphomolybdenum method [19] based on the reduction of Mo(VI) to Mo(V) by the sample analyte and subsequent formation of specific green phosphate/Mo(V) compounds. A 0.3 mL aliquot of extract solution (5-25 mg/mL) combined with 2.7 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was capped and incubated in a boiling water bath at 95 °C for 90 min. Samples were allowed to cool at room temperature and absorbance was measured at 695 nm. For the blank, 0.3 mL methanol/double distilled water was mixed with 2.7 mL of the reagent. A standard curve of Trolox (10-100 µM) was prepared and total antioxidant activity was expressed as µM Trolox equivalent.

Reducing power assay: The reducing power was determined as per standard methodology [20]. Various concentrations (5-25 mg/mL) of methanolic/aqueous extracts (200 µL) were taken and volume was made up upto 1 mL by distilled water, then added 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The resulting mixture was kept at 50 °C for 20 min. Then in the reaction mixture, 2.5 mL of 10 % trichloroacetic acid (w/v) were added and centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) was taken and mixed with 2.5 mL of de-ionized water and 0.5 mL of 0.1 % of ferric chloride. The absorbance was measured at 700 nm; higher absorbance indicates higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated. Butylated hydroxy toluene was used as standard.

Determination of metal chelating activity: The chelation of ferrous ions by mushroom extract was determined using modified method [21]. The sample (0.5 mL) of different concentrations (5-25 mg/mL) was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously, kept at room temperature for 10 min and absorbance of the resulting solution was measured at 562 nm. The metal chelating activity (MCA) was calculated according to the following equation:

$$\text{Metal chelating activity } (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where, A_{sample} is the absorbance of the solution recorded during addition of extract/reference at a particular level and $A_{control}$ is the absorbance of the $FeCl_2$ and ferrozine solution without addition of extract. The extract concentration providing 50 % metal chelation (EC_{50}) was calculated for comparison.

Mineral analysis: The oven-dried mushroom samples were ground to pass through a 0.2 mm sieve for estimation of nitrogen (N) content in Kjeltec 2300 auto-analyzer (Foss Pvt. Ltd). For estimation of phosphorus, potassium, zinc, copper, iron and manganese, sieved mushroom samples were digested with a mixture of nitric acid and perchloric acid in the ratio of 10:4 (v/v) on hot plates sand bath. After complete digestion, samples were cooled at room temperature and appropriately diluted. The phosphorus content was estimated by the ammonium molybdate method [22] and the potassium content by flame photometry [23]. Total zinc, copper iron and manganese were analyzed by atomic absorption spectrometry (AAS vario 6, Analytik Jena).

Statistical analysis: The statistical analyses were performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL). Analyses of variance were performed by ANOVA and significance of each group was verified with one-way analysis of variance followed by Duncan's multiple range test ($P < 0.05$). All determinations were performed in triplicate. For multivariate comparison, principal component analysis (PCA) was used to display the correlations between the various antioxidants and other parameters and their relationship with three different drying methods. Multivariate analysis was carried out using the SAS JMP 9.0 software.

RESULTS AND DISCUSSION

Total polyphenols: Total phenolic contents for the analyzed dried mushroom samples evaluated by the Folin-Ciocalteu method are shown in Fig. 1. Results were expressed as mg GAE/g dry wt. mushroom sample. The amount of total phenolics in solar dried sample was significantly higher (31.4 mg GAE/g dry wt.) and at par with hot air dried sample (28.7 mg GAE/g dry wt.) while it was 27.3 mg GAE/g dry wt. in sun dried mushroom sample. The content of phenolic compounds could be used as an important indicator of antioxidant capacity. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds [24].

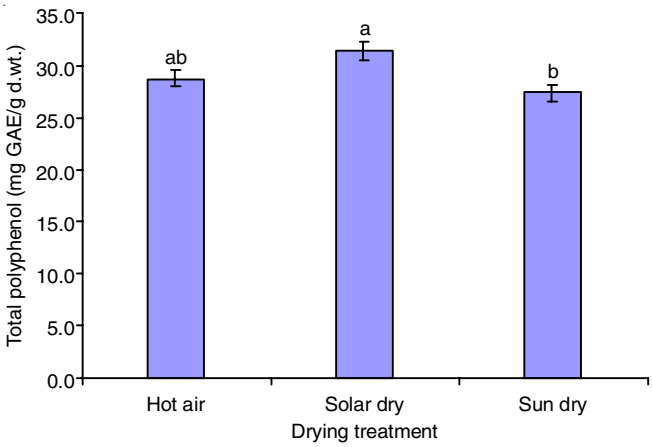

Fig. 1. Total phenolics content (mg GAE/g mushroom) of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error. Samples with the same letters are not significantly different at $p < 0.05$

Radical scavenging activity on DPPH and ABTS: Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The radical scavenging activity (RSA) of dried mushroom samples was tested against the DPPH and ABTS. Radical scavenging activity on DPPH and ABTS of dried mushroom samples varied from 2.19-53.74 and 16.36-94.88 %, respectively (Fig. 2a & 2b) and was found to be concentration dependent. Radical scavenging activity on DPPH and ABTS was higher in solar dried samples (53.74 and 94.88, respectively at 25 mg/mL concentration); however, it was 49.12 and 91.13, respectively at 25 mg/mL concentration for sun dried samples. Scavenging effects of the acetonic, methanolic and aqueous extracts from the fruiting bodies of *P. salmoneostramineus* [25] and *P. citrinopileatus* [26] increased with increasing concentrations. *A. bisporus, P. eryngii, P. ferulae* and *P. ostreatus* fruit bodies scavenged DPPH radicals by 46.6-68.4 % at 5.0 mg/mL [27]. With the presence of radical scavenging activity, consumption of dried oyster mushroom samples might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses. 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain breaking antioxidants [28].

Ferric reducing antioxidant power (FRAP): All the dried mushroom samples showed increased FRAP with the increase in concentration (Fig. 3). At 25 mg/mL concentration, the ferric reducing power of sun dried samples was found to be higher (265.08 μM $FeSO_4$ equivalent) whereas, the lowest FRAP was recorded in hot air dried samples (126.11 μM $FeSO_4$ equivalent at 25 mg/mL conc.). The results revealed that various dried mushroom samples possessed hydrogen-donating capacity indicating the significant reducing power.

Total antioxidant activity: Total antioxidant activities of dried samples showed increased trend with increase in concentration (Fig. 4). Consistently for mushroom samples, solar dried samples showed higher antioxidant activity (384.58 μM Trolox equivalent) at 25 mg/mL concentration, however, it was 378.68 and 372.05 μM Trolox equivalent for hot air and

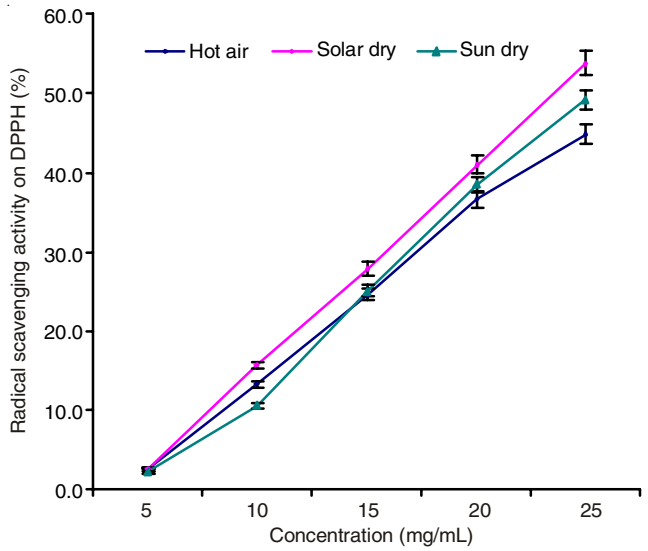

Fig. 2a. Radical scavenging activity on DPPH of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error

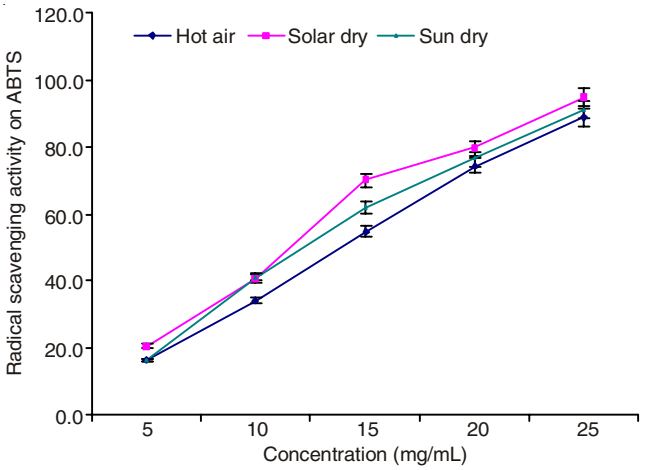

Fig. 2b. Radical scavenging activity on ABTS of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error

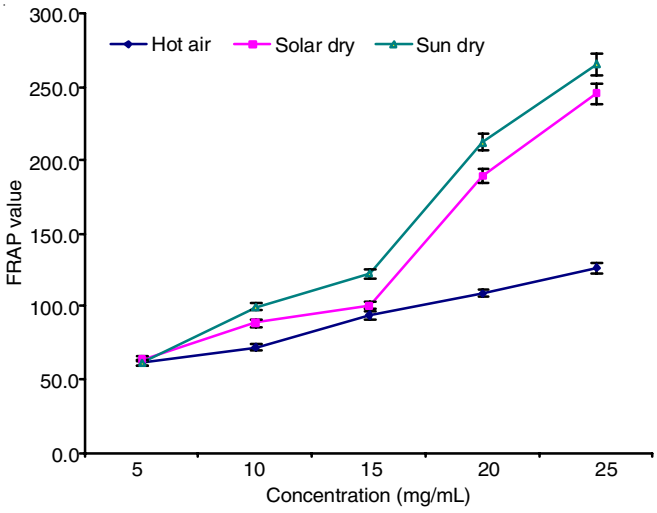

Fig. 3 FRAP value (μM $FeSO_4$ equivalent) of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error

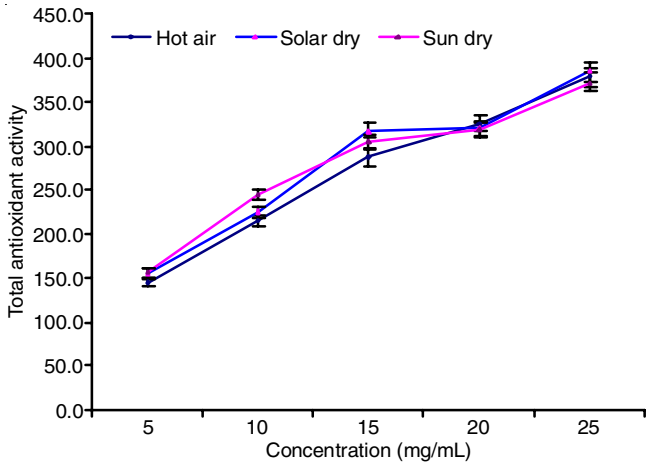

Fig. 4. Total antioxidant activity of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error

sun dried samples, respectively. Increase in antioxidant activities of *Pleurotus citrinopileatus* with increased concentration was also reported [29].

Reducing power: Reducing power of all the dried mushroom samples was excellent and increased steadily with the increased concentration (Fig. 5). The reducing powers of solar and sun dried samples was 0.30 at 25 mg/mL concentration and it was at par with hot air dried sample (0.29). The high reducing power exhibited by the sample might be indicative of the hydrogen donating ability of the active species present in the extracts [30]. Accordingly, all samples might contain higher amounts of reductone, which could react with free radicals to stabilize and block radical chain reactions.

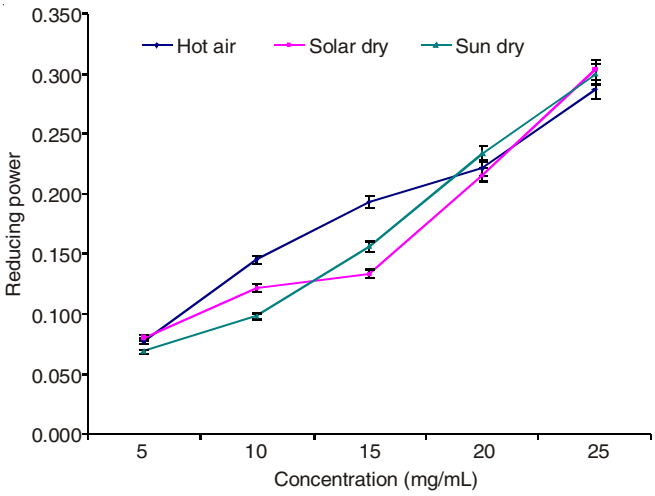

Fig. 5. Reducing power of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error

Metal chelating activity: The metal chelating activity of different dried mushroom samples on ferrous ions increased with increase in concentration (Fig. 6). The chelating activity of hot air dried sample (78.12 % at 25 mg/mL concentration) was higher but at par with solar and sun dried samples (77.50 and 74.37 %, respectively). Chelating ability of *H. marmoreus* (75.6-92.6 %) at 1-5 mg/mL was also reported [31]. Chelating agent may serve as secondary antioxidants as they reduce redox potential and stabilize the oxidized forms of metal ions.

TABLE-1
MINERAL COMPOSITION OF *Pleurotus sajor-caju* AFTER DIFFERENT DRYING TREATMENT

Drying treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Zinc (mg/g)	Copper (mg/g)	Iron (mg/g)	Manganese (mg/g)
Hot air drying	4.58 ± 0.13a	0.79 ± 0.02a	5.01 ± 0.14a	0.092 ± 0.002a	0.009 ± .003ab	0.167 ± 0.05a	0.034 ± 0.009a
Solar drying	4.84 ± 0.13a	0.70 ± 0.01b	5.07 ± 0.14a	0.093 ± 0.003a	0.010 ± 0.003a	0.168 ± 0.05a	0.035 ± 0.002a
Sun drying	4.43 ± 0.13a	0.69 ± 0.02b	4.80 ± 0.13a	0.091 ± 0.002a	0.092 ± 0.003b	0.149 ± 0.04b	0.032 ± 0.009a

All data are means of three (n = 3) independent measurements ± standard error; Within a column, samples with the same letters are not significantly different at P < 0.05.

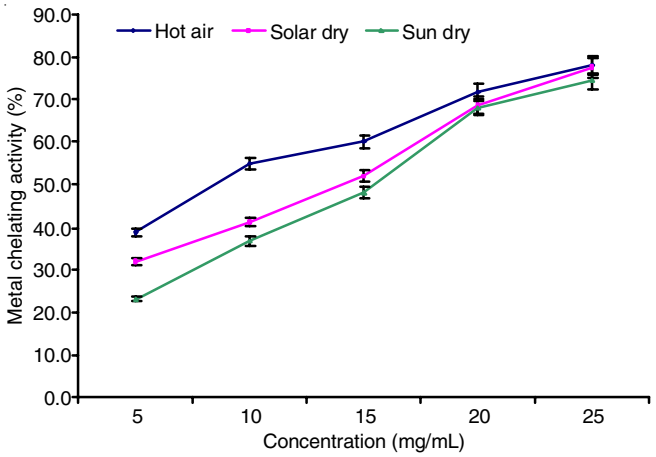

Fig. 6. Metal chelating activity of dried oyster mushroom. All data are means of three (n = 3) independent measurements ± standard error

Since ferrous ions are the most effective pro-oxidants in food system, the high ferrous ion chelating abilities of the dried mushroom samples would be beneficial. This study could provide valuable information to support hot air and solar dried oyster mushroom as an excellent source of antioxidants in human diet.

Mineral composition: All the dried mushroom samples were further analyzed for mineral composition and the data obtained were presented in Table-1. Results showed higher amount of nitrogen (4.84 %), potassium (5.07 %), zinc (0.093 mg/g), copper (0.010 mg/g), iron (0.168 mg/g) and manganese content (0.035 mg/g) in solar dried oyster mushroom, however, significantly higher amount of phosphorus (0.79 %) was found in hot air dried oyster mushroom. Mushrooms are reported to contain some important minerals like Ca, Fe, Zn, P, *etc*. [32,33]. Results obtained for the zinc concentration are within range of 0.03-0.18 mg/g as reported earlier [34]. The results obtained for iron are in agreement with the reported values of 0.01-1.58 mg/g [35].

Multivariate analysis: Principal component analysis (PCA) is a useful statistical technique which has found application in reduction of the original variables (total phenolics, radical scavenging activity on DPPH and ABTS, FRAP, total antioxidant activity, reducing power and metal chelating activity) in a smaller number of underlying variables (principal component) in order to reveal the interrelationships between the different variables and to find the optimum number of extracted principal components. The first principal component (PC1) had the highest eigen value of 20.15 and accounted for 53 % variability in the data set while second principal component (PC2) had eigen value of 17.84 and accounted for 47 % variability. The principal component analysis (PCA) and their correlation are shown in Fig. 7. Among the parameters, radical scavenging activity on DPPH and ABTS were observed on the right upper side of the biplot with high positive loading for both PC1 and PC2, while FRAP was found with slightly lesser positive loadings on the right side of the biplot. This suggests radical scavenging activity on DPPH and ABTS are having positive correlation with solar dried *P. sajor-caju*. Principal component analysis showed positive correlations among the ascorbic acid, metal chelating activity, total phenols and DPPH scavenging activity in different edible mushrooms [36]. The results also showed that sun dried *P. sajor-caju* samples contained higher FRAP, while hot air dried samples showed higher metal chelating activity and total antioxidant activity.

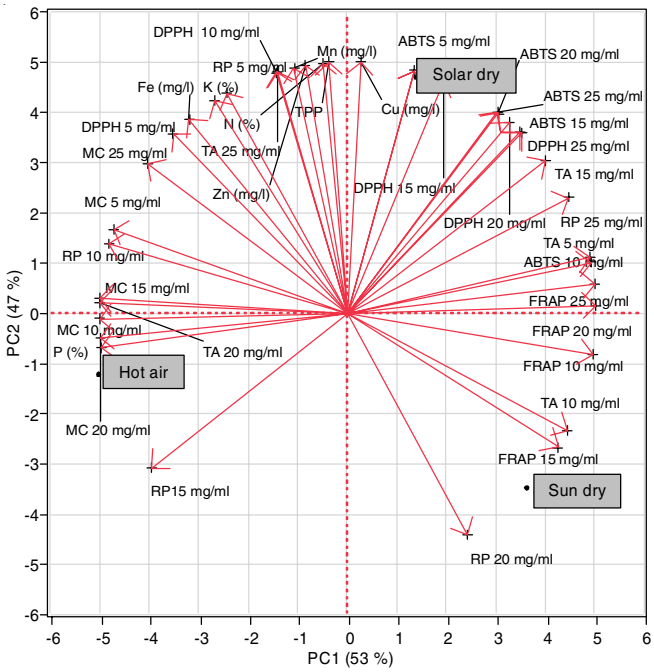

Fig. 7. Multivariate comparison of different drying methods and various parameters using principal component

Conclusion

The oyster mushrooms are very delicate, sensitive and start deteriorating immediately after the harvest; hence mushrooms need to dry in order to extend the shelf life. The results of the present study indicated that different dried oyster mushrooms are rich in different antioxidant activities and minerals. However, solar drying of oyster mushrooms is a practical approach where farmers can add the antioxidant activities and extend the shelf life of mushrooms. Solar dried oyster mushrooms can be incorporated in various recipes for

improving the nutritional status of vulnerable population in north-western Indian Himalayan region.

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