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Comparison of Non-Enzymic Antioxidant Status of Fresh and Dried Form of *Pleurotus florida* and *Calocybe indica*

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Abstract: The modulation of the mushroom used now-a-days in the field of medicine, is the way where this is partly achieved. Among these, the “antioxidants” which are capable of deactivating the free radicals follow either enzymatic or non-enzymatic pathways. The aim of this project is to incorporate the non-enzymatic antioxidant in fresh and dried form of powdered samples of the oyster mushroom (*P. leurotus florida*) and milky mushroom (*Calocybe indica*). This study revealed the presence of non-enzymatic antioxidant in the selected samples and the striking feature of this work is to maximize the usage of mushrooms in the powdered form when there is certain unstable conditions for the usage of mushroom in the fresh form.

Key words: Nonenzymatic antioxidant, oyster mushroom (*P. leurotus florida*), milky mushroom

Introduction

The modulation of immune response by using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations. The basic concept has, however, existed in the ancient vedic scripture, the Ayurveda, and has been practiced in Indian traditional medicine for many centuries this ancient system of medicine evolved in India thousands of years ago, probably represented the first record of scientific medicine in the history of the world.

The two main approaches to illness in Ayurveda are preventive and curative. The major approach to therapy in Ayurveda is one which emphasizes prevention. The second approach is the curative one, i.e., that which attempts to alleviate disease after they have occurred (Upadhyay, 1997).

During the functioning of the immune system such as phagocytosis, reactive oxygen and nitrogen species are generated. If they are left unchecked, they can affect the components of the immune system by inducing oxidative damage. This is more so in the elderly or during inflammation where there is excess generation of these reactive species that can be taken care of by the defenses in the form of “ANTIOXIDANTS”. Dietary supplementation with antioxidants may greatly help in such conditions. Natural compounds from medicinal plants having antioxidants and immunomodulatory activities have potential as therapeutic agents (Janeway and Travers, 1994; Mossman and Coffman, 1989). People have become more health conscious in recent years. One etiologic agent implicated in diseased state is “oxidative stress” which involves excess generation of prooxidants. In a healthy human body the generation of prooxidants in the form of reactive oxygen species (ROS)

and reactive nitrogen species (RNS) are delicately balanced by the antioxidant defenses. (Sies, 1986).

The ROS include the superoxide anions, hydroxyl anions and hydrogen peroxide, singlet oxygen are by products of oxidative respiration and lipid metabolism (Percival, 1996). All oxidants generally influence the redox status, there by protecting cell against Reactive Oxygen Species (ROS) under certain circumstances, while promoting ROS generation in other (Herbet symposium, 1996).

Free radical oxidative stress have been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses. Potential antioxidant therapy, should be therefore include either natural free radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of the antioxidant enzymes. (Bast *et al.*, 1991; Bhattacharya *et al.*, 1991).

India is blessed with varied agroclimate, abundance of agricultural wastes and manpower making it most suitable for the cultivation of all the types of temperate, subtropical and tropical mushrooms. (FAO, 1997).

Among the fungi, mushrooms have been used for untold centuries as food and medicine. Edible and medicinal mushrooms not only convert the huge lignocellulosic biomass waste into human food, but most remarkably can produce notable mycopharmaceuticals, myconutriceuticals and mycosmeceuticals for many years, mankind has benefited from green plants as a source of drugs and herbal remedies. Fungi, on other hand, have not been considered in any significant way. However this is changing rapidly. The prominence of fungi can now be seen increasingly as evidenced by their use as a major source of pharmaceuticals and medicinal foods (Law and Ng, 2001).

Impressed with the voluminous reports on the

applications of mushroom, and to our knowledge so far there are no upto-data to report on non-enzymatic antioxidant levels in the fresh and dried mushroom samples of *Pleurotus florida* and *Calocybe indica*. Mushrooms are highly perishable and get spoiled due to wilting, veil opening, loss of texture, aroma, flavor etc., making it unsuitable for marketing. Drying is the common and most effective method of preservation. Dried mushrooms are convenient for long term storage and transportation. Processing of mushrooms extends the shelf life of mushroom. So the present project work was aimed at analyzing the non-enzymic antioxidants namely Vitamin A, Vitamin C, (Ascorbic acid), Vitamin E (α -Tocopherol) and Reduced Glutathione in fresh and dried samples of Oyster mushroom (*Pleurotus florida*) and Milky Mushroom (*Calocybe indica*).

Materials and Methods

Sample collection: The mushroom samples were collected from Blue hill Mushrooms. Thondamuthur, Coimbatore, India and the specimen sample is preserved in the Biochemistry Department, Dr. N.G.P. Arts and Science College, Coimbatore, India.

Preparation of the sample:

- 1 The fresh mushroom sample was collected, shade dried and coarse powder of the sample was used for the study.
- 2 The fresh mushroom sample was collected, preserved and required quantity was directly used for the assay.

Estimation of vitamin A: Vitamin A from the fresh and dried sample was extracted twice with 10 ml proportions of petroleum ether (40°-60°C). Pooled the extracts and washed thoroughly with water separating the layers using separating funnels. Added sodium sulphate (anhydrous) to remove the moisture. 1 ml of the ether extract was then taken and evaporated to dryness at 60°C. The dried residue was dissolved in 1 ml chloroform and used for estimation. The estimation of Vitamin A in the sample was analyzed using the method of Bayfield and Cole (1980).

Estimation of ascorbic acid: Ground about 1g of the sample and homogenized in 4% TCA up to 10 ml. Centrifuged at 2000 rpm for 10 minutes. The supernatants obtained were treated with a pinch of activated charcoal. Shaken well and kept for 10 minutes. Centrifuged once again to remove the charcoal residue. Noted the volume of the clear supernatants obtained and this supernatant were used for assay. The estimation of ascorbic acid in the sample was done using the method of Roe and Keuther (1953).

Estimation of α -Tocopherol: The tissue were

homogenized in a blender. Weighed accurately 2.5 g of the homogenized tissue into a conical flask. Added 50ml of 0.1 N sulphuric acid slowly without shaking. Stoppered and allowed to stand overnight. The next day the content of the flask were shaken vigorously and filtered through Whatman No.1 filter paper, discarding the initial 10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation.

The estimation of α -Tocopherol in the sample was analyzed using the Emmerie-Engel method, 1938, as described by Rosenberg (1992).

Estimation of reduced glutathione: The samples (1g) were homogenized in 5%TCA to give a 20% homogenate. The precipitated proteins centrifuged down at 1000 rpm for 10 minutes. The homogenate was cooled on ice and 0.1 ml of the supernatant was taken for the estimation. The estimation of reduced Glutathione in the fresh and powdered samples of Oyster mushroom was carried out by the method of Moron *et al.* (1979).

Results and Discussion

The non enzymic antioxidants include Vitamin A, Vitamin C, Vitamin E, and Reduced Glutathione. They scavenge a wide variety of free radicals. Mushrooms are an important source of vitamins. The vitamins of group B are abundant (Mattila *et al.*, 2000, 2001), particularly thiamine, riboflavin, pridoxine, pantotene acid, nicotinic acid, nicotinamide, folicacid and cobalamin as well as other vitamins such as ergosterol, biotin, tocopherols (Mattila *et al.*, 2001). According to USD a National Nutrient Database, Mushrooms are high in protein, vitamins and essential elements including calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese and selenium. Mushrooms are a group of fungi with good source of high quality proteins, rich in vitamins and minerals and its nutritional value equal to meats, eggs and milk with low calorie and cholesterol free. The Non- Enzymic Antioxidants in Milky Mushroom (Fresh and Dried samples) are indicated in Table 1a.

The levels of Non Enzymic antioxidants in Fresh and Powdered samples of Oyster Mushrooms are given in Table 1b.

Vitamin A also acts an neutralizing agent for the free radicals, Vitamin A, a in the fresh and powdered samples of Milky Mushrooms was found to be 0.324 ± 2.51 mg/g (Fresh samples) and 0.215 ± 1.527 mg/g (Powdered samples) and in Oyster Mushrooms, the activity was found to be 0.35 ± 0.03 mg/g (Fresh samples) and 0.276 ± 0.025 mg/g (Powdered samples) mg/g as given in Table 1a and Table 1b. Vitamin A, a fat soluble vitamin is stored in the body and is necessary for clear vision in dim light. Vitamin A maintains the integrity of epithelial tissue (Gopalan *et al.*, 2000).

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Table 1a: Levels of Non Enzymic Antioxidants in Milky mushrooms (Fresh and Powdered samples)

Sample	Concentration (µg)	Vitamin A (mg/l)	Vitamin C (mg/l)	Vitamin E (mg/l)	Reduced Glutathione (nmoles/g)
Fresh	500	0.324±2.51	1.033±0.152	2.933±0.02	0.156±0.025
Powdered	500	0.215±1.527	0.4±0.1	0.80±0.015	0.123±0.015

Values are Mean ± S.D. of duplicates.

Table 1b: Levels of Non Enzymic Antioxidants in Oyster mushrooms (Fresh and Powdered samples)

Sample	Concentration (µg)	Vitamin A (mg/l)	Vitamin C (mg/l)	Vitamin E (mg/l)	Reduced Glutathione (nmoles/g)
Fresh	500	0.35±0.03	0.36±0.02	7.28±0.02	0.176±0.025
Powdered	500	0.276±0.025	0.27±0.025	5.15±0.02	0.123±0.015

Values are Mean ± S.D. of duplicates.

Vitamin A and retinoids, either topically or orally administered, were able to induce complete remission in a high proportion of patients with basal cell and advanced squamous cell carcinoma (Lehninger *et al.*, 1996).

Thus a higher activity was noticed in fresh samples of milky mushrooms than in powdered samples. In Oyster mushrooms a higher activity was seen in fresh samples than in powdered samples.

Vitamin C is regard as the first line natural antioxidant defense in plasma and a powerful inhibitor of LPO (Maxwell, 1995). Vitamin C is a water soluble antioxidant. It acts as a free radical scavenger. It scavenges peroxy radicals (Sies, 1993). Vitamin C protects non-smokers against the harmful effects of ROS from passive smoking (Jacob, 2000). The levels of vitamin C in the fresh and powdered samples of milky mushrooms was found to be 1.033 ± 0.152 mg/g (Fresh samples) and 0.40 ± 0.10 mg/g (Powdered samples) and in Oyster mushrooms it was found to be 0.36 ± 0.152 mg/g (Fresh samples) and 0.27 ± 0.025 mg/g (Powdered samples) as given in Table 1a and 1b. Thus a higher activity was notices in fresh samples of milky mushrooms than in powdered samples. In Oyster mushrooms, fresh samples have a higher level of Vitamin C when compared with powdered samples.

Vitamin E is a fat soluble vitamin mainly formed intercalated within the membrane. It prevents the attack of reactive oxygen species of the membrane PUFA (Sies, 1993). Vitamin E (α -tocopherol) is probably the most important lipid-soluble antioxidant protecting membranes, lipids and lipoproteins (VanBakl *et al.*, 2000). Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels has been shown to enhance immune response and resistance to diseases (Bendich, 1997). Many studies have suggested that high intake of Vitamin E may slow down the development and progression of atherosclerosis. Some clinical trials also reported beneficial effects of Vitamin E supplementation in the secondary prevention of cardiovascular events (Meydani, 2000). The levels of Vitamin E in the fresh and

powdered samples of milky mushrooms was noted to be 2.933 ± 0.02 µg/g (Fresh samples) and 0.80 ± 0.015 µg/g (Powdered samples) and in Oyster mushrooms it was found to be 7.28 ± 0.02 µg/g (Fresh samples) and 5.15 ± 0.02 µg/g (Powdered samples) as given in Table 1a and 1b. Thus, the levels of α -tocopherol was found to be higher in powdered samples of milky mushrooms than in fresh samples. In Oyster mushrooms, fresh samples have a higher level of vitamin E when compared to powdered samples.

The glutathione (GSH) is the most abundant non protein thiol, GSH / GSSG pair forms the major intracellular redox system. Functions of GSH in reductive processes are essential for the synthesis and also the degradation of the levels of proteins, formation of DNA, regulation of enzyme activities and protection of the cells against ROS and free radicals produced even in normal metabolism (Gul *et al.*, 2000). GSH in the fresh and powdered samples of milky mushrooms was noted to be 0.156 ± 0.025 (nmoles/g) (Fresh samples) and 0.123 ± 0.015 (nmoles/g) (Powdered samples) and in Oyster mushrooms it was found to be 0.176 ± 0.025 (nmoles/g) (Fresh samples) and 0.123 ± 0.015 (nmoles/g) (Powdered samples) as given in Table 1a and Table 1b. Thus, the levels of GSH was found to be higher in fresh samples of milky mushrooms than in powdered samples. In Oyster mushrooms, the levels of GSH was found to be higher in fresh samples than in powdered samples.

Conclusion: As coated by Hema *et al.*, TNAU Coimbatore, India, dietary intake with addition of Antioxidants may provide great relief to the problem caused by ROS. Mushrooms preserved by drying have a good flavor, prevents microbial deterioration and enhances the appearance. The moisture content of dried mushrooms is in the range of 10-15 % (d.b.) and has superior quality of protein content varying from 25-35 % (d.b.).

From the present study, it could be concluded that the both fresh and dried samples of Milky mushrooms and Oyster mushrooms posses non-enzymatic activity.

Though, the fresh sample contains higher activity compared to dried samples, since the shelf life of fresh mushroom may be extended by refrigeration at 4°C, but its storage is not always effective. So in order to maximize the usage of mushroom in our day to day life, we could preserve the mushrooms in dried form. Though the activity is low compared to fresh mushrooms, significant difference in non-enzymatic was not noticed in fresh and dried samples. The utility of mushroom could be enhanced by preserving them in dried form and at the time of surplus production. When there is devoid of production in the off season especially during summer, these dried samples could be used. Thus the value of mushroom could be greatly enhanced since mushroom are described as "Precious Pearls of Cookery" by Hema *et al.*, (2002) TNAU.

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