Studies on Biofortification of Selenium in Edible Mushrooms

A thesis submitted in fulfillment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY

in

Department of Biotechnology

by

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May 2014

Certificate

It is hereby certified that the thesis **"Studies on biofortification of selenium in edible mushrooms"** which is submitted by Miss **Poonam Bhatia (Regd. No. 900900002),** in fulfillment of the requirement for the award of the degree of ***Doctor of Philosophy*** in the Department of Biotechnology, Thapar University, Patiala, India, is a record of the candidate's own independent and original research work carried out by her under my supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree in India or abroad.

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Declaration

I hereby declare that the work which is being presented in the thesis **"Studies on biofortification of selenium in edible mushrooms"** submitted by me for the award of the degree of ***Doctor of Philosophy*** in the Department of Biotechnology, Thapar University, Patiala, India, is true and original record of my own independent and original research work carried out under the supervision of Dr. N. Tejo Prakash, Professor, School of Energy and Environment, Thapar University, Patiala, India and Dr. Ranjana Prakash, Associate Professor, School of Chemistry and Biochemistry, Thapar University, Patiala, India. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree in India or abroad.

Date: 16/7/2014

Place: Patiala

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Acknowledgement

At the very onset and above all I must bow with all the humility before the divine power (Satguru baba ji) who has bestowed me with the requisite intelligence health and above all the will to carry out to consummation the stupendous task of investigation. I also thank him for bringing in contact with learned team of guides as well as loving and caring friends all of whom have contributed to my success.

I feel privileged to express my dense sense of gratitude, indebtedness to my respected and worthy supervisor ***Prof. N. Tejo Prakash,*** *Professor, School of Energy and Environment , Thapar University, Patiala, India and* ***Dr. Ranjana Prakash,*** *Associate Professor, School of Chemistry and Biochemistry, for their valuable guidance, keen interest, constant and vital encouragement, constructive criticism, patronage and dedication for research rendered to me during my work as well as in preparation of manuscript, which led me with perfection to achieve my goal. I extend my heartfelt thanks to them forever throughout my life.*

I would like to thank the rest of my doctoral committee, ***Dr. Sanjai Saxena (****Associate Professor, DBTES, TU) and* ***Dr. Manmohan Chibber****, (Assistant Professor, SCBC, TU), for their encouragement, insightful comments, and relevant mid-course suggestion.*

I deem it a pleasure to express my regards and gratitude to ***Dr. Dinesh Goyal (****Professor and Head, DBTES, TU),* ***Dr. A.S.Reddy*** *(Head, SEE, TU) for their kindness, support, professional guidance and valuable advice in many different ways and* ***Dr. P.K.Bajpai****, Dean (Research and Sponsored Projects) for providing help and support during the course of work.*

I am thankful to ***Dr. S. K. Pandey*** *(Assitant Professor in SCBC, Thapar University, Patiala) for giving me all the valuable tips and suggestions to sort out the problems in my experimental work.*

Sincere thanks to ***Dr. Harpreet Singh Sodhi*** *(Senior Mycologist) in the Department of Microbiology, Punjab Agricultural University, Ludhiana for being extremely supportive for providing facility to cultivate mushrooms , without his help, I could not have reached my goal. I wish to convey my regards and gratitude to him for his advice, sharing his knowledge, and encouragement which have helped me in learning the mushroom cultivation.*

My warm thanks are due to all faculty members of Department of Biotechnology and School of Energy and Environment, TU, Patiala, for their meticulous guidance, keen interest, invaluable suggestion and constant support during the course of my study. The personal and professional guidance that I received from them would be cherished lifelong. Special thanks to ***Dr. Anita Rajor,*** *for being a motherly figure to me and for her guidance and suggestions.*

I express my regards and grateful to my seniors ***Dr. Saurabh Gupta, Dr. Neetu Sharma, Dr. Satnam Singh Aulakh*** *and my lab mates* ***Mr. Sumit Kumar Jaiswal, Ms. Noorpreet Dhanjal, Ms. Jashan, Mr. Anirudh Sharma****,* ***Ms. Divya Singhal, Ms. Chandni Bansal, Ms. Shruti Sharma, Ms. Vasudha and Ms. Harneet Kaur*** *for providing keen interest, unfailing support, inspiration, critical observations and ingenuous suggestions for my research work.*

*I would like this opportunity to express my heartfelt thanks to my friends****, Ms. Pankil Singla, Ms. Gurdeep Kaur, Ms. Mandeep Kaur, Mrs. Tejinder Kaur , Ms. Baljinder Kaur*** *for their invaluable smiles and friendship during the course, which has motivated and encouraged me for my research work. I also thank those who could not find a separate name but helped me directly or indirectly.*

*I extend my thanks to our technical assistants **Mr. Baban** and **Mrs. Lalita** at DBT, who had provided me all the help and support for my research work.*

*I would like to convey my thanks to **Mr. Ashwani Aggarwal Mr. Jain** and **Mr. Vijay** for their cooperation.*

*For all the sacrifices that **my dear parents** so selflessly made, words at my command are inadequate in form and spirit to convey the depth of feelings towards them for showering their blessings, flourishing, inspiration, encouraging support which brought me up to this level. Words are too less to express my love towards my younger brother **Shekhar** and sweet sister **Aanchal** for their loving affection throughout.*

*I wish to express my gratitude and thanks to **UGC** for proving me financial support in the form of **Rajiv Gandhi National fellowship** and **Thapar University, Patiala,** for providing me good infrastructure facilities to help accomplish my work.*

Date:

Place: Patiala *Poonam Bhatia*

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1.0 Introduction

Selenium is one of the rare elements on the earth crust, being about 70th in abundance. The evidence of Se as an essential element gathered over time through large number of observations on the nutritional importance of Se (Schwarz and Foltz, 1957; Combs, 1990; Whanger, 2002). The atomic weight and number of selenium is known to be 78.96 and 34.0 respectively (Table 1.1). Selenium can occur in four different oxidation states that include elemental (0); selenide (-2); selenite (+4) and selenate (+6). Organoselenium compounds such as selenoaminoacids represent important bio-available forms of Se with selenomethionine being more bioavailable than selenocysteine (Figure 1.1). This aspect is governed by number of factors that include soil texture, pH, redox potential and organic composition, along with these, other factors like artificial fertilization, rainfall rate, presence of competitive ions and human activities are also considerable (Seby et al., 1997). In nature, selenium completes its biogeochemical cycling through microbes mediated redox reactions leading from all of its oxidation states (Dowdle and Oremland, 1998). Many micro-organisms especially bacteria (Fellowes et al., 2013) and fungi (Vetchinkina et al., 2013) are known to biotransform excessive amount of selenium to its elemental state thereby resulting to detoxification.

Selenide and elemental selenium occur in acidic, reducing and organic rich environment. The oxidized forms such as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are more soluble and easily absorbed by plants, whereas Se^{2-} and Se^0 are less available (Fishbein, 1991; Banuelos et al., 2002). Volatile compounds such as dimethylselenide (DMSe) and dimethyldiselenide along with few toxic methylated species trimethylselenonium ion (TMSe), are found to be less toxic species of selenium (Lemly, 1997; Pyrzynska, 1998). Selenium possess six naturally occurring stable

isotopes: ^{74}Se, ^{76} Se, ^{77}Se, ^{78} Se, ^{80} Se and ^{82} Se. Due to distinctive physical properties of selenium, it has various uses in manufacturing processes such as in manufacture of glass and pigments, photoreceptors, semiconductors, as well as in the dry photocopiers. Apart from manufacturing, considerable amount of Se is used as dietary supplement for humans as well as farm animals.

Table 1.1. Physical and chemical properties of selenium (Se) (Zingaro and Cooper, 1974)

Important Properties of Selenium	
Periodic position	Group VIA, Period 4
Atomic number	34
Atomic weight	78.96
Atomic radius (uncharged)	215 pm
Stable isotopes	6
Radioactive isotopes	14
Hardness (relative units)	2
Allotropic forms	Trigonal (grey), α monoclinic (red), β monoclinic (red) Red amorphous, black amorphous, vitreous (black)
Melting point (amorphous)	323 K
Boiling point	958 K
Oxidation states	-2, 0, +4, +6
Electronic configuration	2-8-18-16
Electronegativity (Pauling scale)	2.54
Entrophy	10.15 cal g/atom
Enthalpies	
Fusion	5.10 kJ/mol
Vapourization	26.32 kJ/mol
Bond energy (Se-Se)	44 kcal/mol
Self diffusion coefficients (Trigonal)	3.8×10^{-12} cm^2/s at 308 K
Standard reduction potentials	
$Se + 2e^- = Se^{2-}$	0.78 V
$Se + 2H^+ + 2e^- = H_2Se$ (aq.)	0.36 V

Although Se was known for its toxic nature for many decades, its essentiality as a micronutrient for health is gaining ground extensively. Se is a well reported element for anti-oxidant properties in humans and animals. A vast section of world's population has sub-optimal

Se-intakes; therefore they are exposed to increased risk of many diseases such as cancer, heart and viral diseases (Combs, 2001).

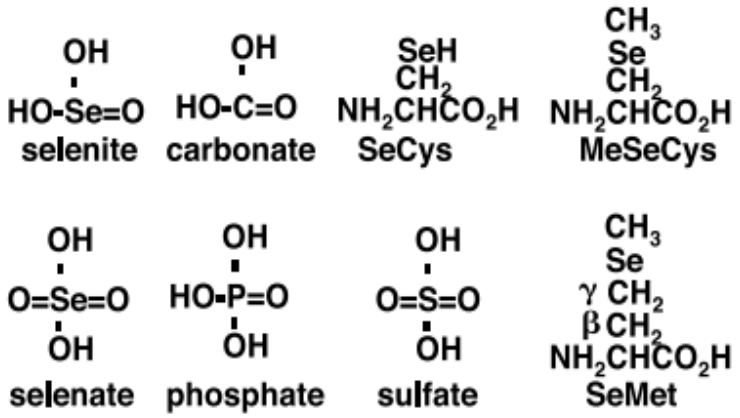


Figure 1.1. Chemical configurations of inorganic selenium compounds, the related oxyanions, and organic selenium compounds (MeSeCys - Se-methylselenocysteine; SeCys – Selenocysteine; SeMet – Selenomethioneine) (Suzuki, 2005)

1.1 Selenium in environment

1.1.1 Global distribution

Selenium is known to be a rare element on earth's crust with average concentration of only 0.05mg/kg in igneous bedrock (Krauskopf, 1982). The limited resources of this element are due to the fact that Se does not possess ores from which it can be mined as primary product (STDA, 2006). In general, there is uneven distribution of selenium on surface of earth but the typical range of distribution of selenium on earth's surface is found to be from zero to 1250 mg/kg. The most common oxidized forms of selenium (selenite and selenate) present in soil may also be present in sedimentary rocks. Moreover sedimentary rocks especially shales and coal contains high Se (average) concentration than igneous rocks (Krauskopf, 1982).

The seleniferous/non-seleniferous status of soil can be classified depending on the selenium level of non-accumulators grown on that soil. Generally, the soil with concentration of selenium as low as 0.1-0.5mg Se/kg (Ravikovitch and Margolin, 1957; Dhillon et al., 1992) is considered seleniferous as forages grown on them contain > 4mg/kg which is the maximum permissible level for human consumption. On the other hand, consumption of feed containing Se < 0.05-0.1 mg Se/kg may lead to deficiency disorders in animals and humans.

Areas with unusually high Se concentrations include Western United States with maximum Se content of 98 mg Se/kg (Rosenfeld and Beath, 1964), San Joaquin valley of California, USA (0.28-2.32 mg/kg) (Severson and Gaugh, 1992) and plains of China containing total Se concentration of ≥ 3 mg/kg in soil and 42.9 μg/kg in water soluble fractions (Tan et al., 1994). Similarly, Se in various soils of India such as Punjab, Haryana and Sub–Himalayan regions of West Bengal are in the range of 0.23 to 10 mg Se/kg (Singh and Kumar, 1976; Dhillon et al., 1992). Some parts of world like Denmark, Finland, New Zealand, Eastern and Central Siberia, parts of China, Central Africa, Northern Korea, Nepal and Tibet are known to have a low Se-soil content (Oldfield, 1999).

1.2 Selenium essentiality and biological profile

The investigation of trace elements is necessary from the view point of nutrition as well as environmental analysis, since at certain steps of chain of nutrition, the quantity of trace elements required for normal vital function may get affected to a significant extent. With respect to health of both animals and humans, selenium in particular is established as an important trace element as it has two faces i.e. it acts as nutrient as well as a potent toxin.

The World Health Organization report advises a Se intake of 55-65 µg/day as the average intake level needed to ensure meeting normative requirements of healthy adults. Similarly, the upper tolerable range for adults has been proposed to be 400 µg/day (FAO/WHO, 2002). Selenium in diet may behave as two sided coin i.e. consumption of food containing < 0.1 µg Se/day leads to deficiency disorders, whereas consumption of food containing > 1 µg Se/day may result in toxicity. The amount of selenium in environment is getting increased due to human activities which are furthur dependent on relatively narrow range between essentiality and toxicity.

Selenium is involved in biological functions through facilitating normal groeth and development at trace concentration and maintaining homeostatic functions at moderate concentrations (Hamilton, 2004).

1.2.1 Biological profile of selenium

Selenium plays an important role in conducting various antioxidant reactions in humans as well as animals. Biochemically, Se is an integral component of antioxidant enzyme glutathione peroxidase (GSHPx), which in combination with other enzymes such as catalase (Cat), superoxide dismutase (SOD) as well as vitamin E, protects the cellular components from oxidative damage by checking peroxide accumulation in tissues.

The biological effects of selenium are exercised through various selenoproteins. Main selenoproteins recognized include classes of (glutathione peroxidases (GPx), thioredoxin reductase, iodothyronine 50-deiodinases (IDI), selenoprotein P (SePP) sperm capsule selenoprotein and selenoprotein W) (Burk and Levander, 1999; Holben and Smith, 1999). The glutathione peroxidases group furthur consists of cellular glutathione peroxidases (GPX1) and

phospholipid hydroperoxide glutathione peroxidase (PHGPx: GPx4). Based on location of selenocysteine in the selenoprotein domain, these are divided into two sections. First group of selenoproteins include glutathione peroxidases, selenoprotein W and P, wherein selenocysteine is located at N-terminal of domain. On the other hand in second group, selenocysteine is present at C-terminal in the function domain. Till date, four different forms of mammalian glutathione peroxidases (GPx1, GPx2, GPx3 and Gpx4) have been recognized. They are known for their active antioxidant role in protecting cellular components from oxidative damage by acting as catalyst in reactions leading to reduction of hydrogen peroxides through oxidation of reducing glutathione (GSH) to oxidized glutathione (GSSG). Selenoproteins such as formate dehydrogenase from *Escherichia coli* (Zinoni et al., 1987), contain selenocysteine residues that are important for catalysis. Selenium shares similar chemical properties with sulphur, hence many enzymes cannot distinguish between the two (Stadtman, 1974) however, Selenophosphate synthetase and selenocysteine lyase strictly distinguish between the selenium and sulphur (Veres et al., 1994). Selenocysteine lyase shares sequence similarity with NifS, a cysteine desulpharase (Mihara et al., 2000). Selenoprotein P, the major plasma selenoprotein has role in metal detoxification as it contains high selenocystine and histidine content. During Se deficiency, the level of selenoprotein P decreases in humans (Burk et al., 2003). Thioredoxin reductase is widely known redox selenoprotein that assists many redox-dependent processes. It is one of the proteins involved in the repair mechanisms essential for DNA synthesis (Tapiero et al., 2003). Many researchers have carried out extensive studies and reported beneficial effect of supplementation of dietary Se in prevention and cure of inflammatory diseases resulting from oxidative stress (Prabhu et al., 2002; Chi-Chen et al., 2009; Desai et al., 2010; Gandhi et al., 2011).

Selenium plays a crucial role in various mechanisms such as thyroid metabolism, immune function and redox reactions (Rayman, 2000). Due to high antioxidant status and active role of Se in metabolism; its deficiency will definitely lead to adverse conditions in human and animal health. Selenium deficiency in animals causes various diseases such as growth suppression and reproductive impairment. Several strategies such as selenium fortification of soil and selenium enriched animal feed have been followed by producers to ensure that animals obtain adequate amount of selenium (Reilly, 1996). Rarely seen in humans, yet Se deficiency appears as an endemic cardiomyopathy in adolescent years, the disease being recognized as Keshan disease (Keshan Disease Research Group, 1979 a,b). It has also been recognized in Se-deficient areas of China (Levander, 1987), in the form of Kashin-Beck disease. Kashin-Beck disease, which is characterized by chronic disabling degenerative osteoarthritis reported to affect about millions of children in China, North Korea and several parts of Russia (Peng et al., 1992). Many chronic diseases such as cancer, cardiovascular diseases, asthma and many others have been associated with low Se status (Neve, 1996; Ip, 1998; Rayman, 2000; Brown and Arthur, 2001). Optimum concentration and activity of selenoproteins is required for prevention of Se-deficiency related diseases. Diet devoid of selenium affects many biological functions as it is dependent of function and metabolism of Se-proteins, therefore the risk of getting exposed to many diseases such as cancer and cardiovascular diseases increases (Navarro-Alarcon and Lopez-Martinez, 2000). A fatal form of cardiomyopathy prevalent among children in China, known as Keshan disease was associated with very low levels of selenium in soil (Li, 1985; Levandar and Beck, 1997; Xia et al., 2005).

Recent findings of human intervention trails (Clark et al., 1996, Yu et al., 1997) have inspired interest in a cancer preventive role of selenium. Apart from its anti-cancer activity,

selenium has also been reported to have an anti-viral effect (Baum et al., 1997; Yu et al., 1997; 1999). Patients undergoing chemotheraphy appears to exhibit immune-stimulating effects, when supplemented with selenium.Various metabolites of selenium results in normal functioning of cellular growth only through increased levels of enzymes such as glutathione peroxidase and thioredoxin reductase (Birringer et al., 2002). Increased Se status is also beneficial to corticoid-dependent asthmatics, as it is associated with decrease in use of cortisteroids in such cases (Gazdik et al., 2002).

In contrast to a variety of beneficial properties, recent epidemiological studies have reported that supranutritional selenium intakes along with high plasma selenium concentrations increase the possibility of developing Type 2 diabetes (Steinbrenner et al., 2011). These observations thus inferred that high selenium diet could enhance the rate of getting affected with diabetes; however, at the nutritional level; the effect of selenium is likely to be very different from the supranutritional level.

1.3 Selenium in seleniferous region- Environment, health and economic perspective

The environmental effects of seleniferous regions around the world have been observed in animal and human health. The geological effect of seleniferous regions on animal health have been observed in Queensland, Australia, where seleniferous limestones and shales of the tambo formation caused selenium toxicity symptoms in livestock grazing in the area (Oldfield, 1999). Another well reported incidence of Se toxicity through water sources in animals have been recorded in at Kesterson reservoir, California, USA (Jacobs, 1989; Tokunaga et al., 1996; Wu et al., 2000) where the developmental deformities in birds included missing eyes, beaks, wings, legs and hydrocephaly.

For the past few years, the attention of several workers has been engaged in the study of the role of Se in Indian soil and plant and its impact on human and animal health. The soils can be classified as seleniferous or non-seleniferous depending on the Se level of non-accumulators (cultivated agricultural crops) grown on that soil. The soils containing as low as 0.1-0.5 mg Se/ kg (Ravikovitch and Margolin, 1957; Dhillon et al., 1992) are considered as seleniferous because the forages grown on such soils contain 0.4 mg Se/kg, i.e., the maximum permissible level for animal consumption. In India, the Se toxic sites identified included parts of Northwest region of Punjab (Dhillon and Dhillon, 1991), Haryana (Arora et al., 1975) and some locations in North-eastern states of Assam and Meghalaya (Dey et al., 1999). Among all the locations identified, agriculturally rich belt along the border of Nawashahr-Hoshairpur districts of Punjab has gained prominence due to extensive research and agri-extension activity (Dhillon and Dhillon, 2003a). The affected villages, covering over 1000 acres are Barwa, Bhano majra, Sikandarpur, Rakkar, Simbli, Jainpur, Mahenpur and Nazarpur (Dhillon and Dhillon, 1997). Preliminary report on chronic Se toxicity as a cause of hoof and horn diseases in livestock in seleniferous regions of Punjab was given by Gupta et al. (1982). Dhillon and Dhillon (2003a), reported that Se content in hair and nail of intoxicated people was 8-9 times higher than normal people. Limited observations on human population in Se-rich regions of Punjab state had also shown signs of toxicity with selenosis in humans, mainly due to consumption of grains and vegetables harvested from Se-rich agricultural soils (Hira et al., 2003). In general, the common features of selenium toxicity includes, loss of nail and hair, nausea, diarrhea, garlic odour on breathe, mottled teeth and abnormalities of nervous system (Surai, 2006). Toxicity to plants was seen in wheat, sugarcane that exhibited papery snow white chlorosis of leaves with pinkish coloration on the sheath and lower surface of leaves (Dhillon et al., 1992).

1.4 Selenium mobilization and biofortification

Although, it has been known since late 1800s that edible flora can accumulate extraordinary levels of metals and clean-up affected environments, the use of these systems as nutrient-supplements has been very limited (Guerinot and Salt, 2001). As many of the metals that can be hyperaccumlated are also essential nutrients, edible plants that accumulate elements such as selenium, in areas that are mineral deficient, can definitely serve as a natural source of mineral supplements in case of animals as well as human beings. From many centuries, plants have been serving as the main source of food, feed and fiber to society. The concept of replacing plants with chemical or nutrient factories for supplying the directly needed macro and micro-elements is drawing significant attention towards biotechnology-based, nutritionally fortified food. Wheat, pulses and certain vegetable crops (among plant foods) are rich sources of selenium.

1.5 Biological properties of Se enriched crops

The increasing knowledge of the effects of selenium containing biological complexes as anticarcinogenic agents, in recent past, has generated immense interest amongst researchers to search for the possible suitable methods of delivery to the human population. Investigation of supplementary methods for quickly delivering these defensive agents through the food system is an apparent approach to be followed (Whanger, 2002). Among most popular selenium supplements, Se- enriched yeast is most widely available to the general public; however Se-enriched garlic has been reported to be two times more effective as Se-rich yeast in the inhibition of tumor growth (Ip et al., 2000). Similarly, Se- enriched broccoli florets (Finley et al., 2000, 2001; Davis et al., 2002) and enriched broccoli sprouts (Finley et al., 2001), are reported to reduce colon tumours in rats. Following a similar paradigm, reports by Bhatia et al. (2013) and

da Silva et al. (2010) indicated that the Se-enriched mushrooms can be considered as an alternative Se food source for humans, due to their high bioavailability. However, there are limited reports on such applications, including bioavailability and bioaccessibility, associated with Se-enriched mushrooms, despite these food systems being known for their health related properties.

1.6 Agri-residues as source of selenium

A prominent characteristic of the agricultural sector is that the by-products and wastes greatly exceed the harvestable or marketable components. Now-a-days in developing and developed countries, agricultural waste is generated in billons of tons each year. Agricultural residues include all leaves, straw, husks and barbajo (leaves and stalk left in the field after harvesting). The wastes consist largely of lignocellulosic materials, an abundant and renewable source of carbohydrates that can be converted into added value products. In agriculturally rich states like Punjab in India, with major produce in the form of wheat and rice, the agricultural activity generates about 23 and 17 million tons (on annual basis) of rice straw and wheat straw, respectively. Among the crop plants, wheat, maize, pulses and certain vegetable crops grown in the region have been found to hyperaccumulate significantly high Se levels (Dhillon et al., 1991; Sharma et al., 2007, Cubadda et al., 2009).

1.7 Mushrooms as selenium mobilizers

Since thousands of years, mushrooms have been appreciated in the human diet. Although mushrooms have long been appreciated because of their flavour, texture and medical properties, yet the nutritional importance is still being explored. Consumption of mushrooms has been markedly increased worldwide and it involves a variety of genera such as *Pleurotus* (oyster

mushroom), *Agaricus* (button mushroom), *Boletus* (Porcini), *Ustilago* (Huitlacoche), *Ganoderma,* etc. Worldwide annual production of mushroom is found to be 61.6 lakh approximately. On dry weight basis, fruiting of mushroom contain nearly 39.9% carbohydrate, 17.5% protein and 2.9% fats (Latiff et al., 1996) and are found to be an excellent source of Vitamin B_2, niacin and folates. In comparison to other crop plants, mushrooms are potential sources of K, P, Zn and Cu (Matilla et al., 2001). Thus, fungi might exhibit an efficient system that readily captures trace elements from the growth medium (Lepsova and Mejstrik, 1998). Mushroom species grow and yield on a spectrum of agricultural residues and by-products, such as paddy, wheat, barley, sunflower, maize and weed straws, jowar and bajra stalks, ground haulms, pod shells, cotton wastes, mango stiff, jute coir pith, sugarcane bagasse, water hyacinth, rubber wood dust and tree leaves (Khanna, 2003).

In general, many edible mushrooms are reported to be accumulating selenium (Anderson et al., 1982; Michelot et al., 1998; Ogra et al., 2004; Falandysz, 2008; Coasta-Silva et al., 2011; da Silva et al., 2012). Apart from edible mushrooms, appreciable accumulation of Se (1-7µg/g) has been observed in poisonous, inedible mushroom (*Amantia*), wherein the content in respective fruiting was 3-6 folds higher than soil (Byrne et al., 1976).

Extensive studies are carried on Se uptake by edible and non-edible mushrooms cultivated on substrates supplemented with Se exogenously; there is limited evidence on Se uptake and biological properties of the Se fortified mushrooms. The earlier observations reported by various researchers also show that Se uptake and speciation studies were carried out only in mushroom cultivated on substrates enriched with Se through its supplementation in the growth or aqueous media. To the best of our knowledge, no study is available on accumulation and speciation of Se in mushrooms grown on agri-residues hyperaccumulated with Se through natural processes.

Keeping this in view, the work presented here has been carried out on Se-rich mushrooms cultivated on Se-rich residues from seleniferous sites. Moreover this study is beneficial in terms of environmental aspects as the waste agri-residues have efficiently been used for cultivation which otherwise are burnt by farmers leading to environmental concerns.

2.0 Review of Literature

Selenium has been a topic of immense research, since 1817, when it was discovered by Jons Jakob Berzelius. Selenium is ranked as 34th element in periodic table. It is a non metal with atomic number 34 and mass 78.96. Selenium has four common oxidation states i.e. elemental Se (0); selenide (-2); selenite (+4) and selenate (+6), which are available in environment. The environmental conditions such as redox potential, determine the identity and magnitude of all the species belonging to various oxidation states in soil. Higher oxidation states of selenium are encouraged in well aerated and alkaline soils, whereas lower oxidation states are favored in acidic and poorly aerated soils (Marian, 1984). In surface waters, selenate and selenite are the most common chemical forms. In general, selenate and selenite have great affinity to be absorbed by soil particles and hence their mobility is reduced to a greater extent, although they are appreciably soluble in water. Elemental Se (0) is usually incorporated in soil particles and exists in crystalline form. Selenium, in particular, is concentrated in the soils of the dried regions where the soils tend to be more alkaline (Sharma and Singh, 1983). Earlier selenium was known for its toxic profile as reported in China (as hoof disease later named as selenium toxicosis) (Oldfield, 2006) and in 1930's this element was found to be responsible for several health ailments. Schwarz and Foltz (1957), reported that during Vitamin E deficiency, selenium supplementation prevents necrosis in liver of rats. Therefore, it is necessary to deal with and observe the relationship between the content of selenium in soil followed by its absorption and accumulation in plants and subsequent transmission to higher levels of food chain as they are directly linked with Se status in terms of deficiency and toxicity.

2.1 Distribution of selenium in environment

Selenium is relatively continuous in environment through processes like volcanic eruptions, fossil fuel combustion, sea salt spray, incineration of municipal waste, forest wild fires, soil leaching, weathering of rocks, transportation of ground water, uptake and release by plants and animals. Selenium is being continuously added to environment (Nriagu and Pacyna, 1988; Mayland et al., 1989; Nriagu, 1989; Dhillon and Dhillon, 2001).

2.1.1 Natural sources

Among other elements, selenium is found to be the most dispersed element on earth, ranking 69th in order of abundance and ranging from 0.03 - 4.08 mg/kg. It occurs in minute amounts in all materials of the crust, but is rarely concentrated in any of the materials in amounts above 100 mg/kg. Rocks serve as primary resource of selenium in terrestrial system (Fleming, 1980; Christophersen et al., 1995). The selenium concentration of rocks varies in different geological formations in different beds of the same formation and even in different parts of the same bed. Tamari et al. (1990), found that sedimentary rock contain Se upto concentration of 0.08 mg/kg (mean value). On an average, igneous, volcanic and phosphatic rocks contain total selenium concentration of 0.35 and 1 mg/kg (Charter et al., 1995; Fordyce, 2005). This is likely due to transfer of volatile forms of Se to the environment (atmosphere and lithosphere) through volcanic activities. Selenium is also available through some minerals such as chalcopyrite, pyrite and other metallic sulphides (Neal and Sposito, 1989; Masscheleyn et al., 1991). High levels of selenium are found in soils derived from cretaceous shales with an average concentration of 4.5 mg/kg (Lakin, 1972) and in organic rich carbonates of Precambrian era in China (Inhat, 1989).

Each of the processes such as sea salt spray, volcanic emanation and suspended dust particles seem to account for 10% of total Se emitted to the atmosphere from natural sources. The major Se form emitted as a result of volcanic activity and fossil fuel burning, as determined from thermodynamic calculations and chemical characteristics, is elemental Se (Suzuki, 1965; Andren et al., 1975). Volcanic rocks such as basalts and rhyolites possess very low Se levels as Se has the tendency to escape at high temperature during volcanic activity (Fleming, 1980; Neal, 1995; Jacobs, 1989). Marine biogenic sources can account for 60–80% of the total Se to be released from natural resources on annual basis. Biological release of dimethylselenide (DMSe) into the atmosphere can possibly be ranked as a vital factor in the Se distribution (Doran and Alexander, 1977). In addition to this, Se has been detected in remote regions of the world such as Antarctica (Zoller et al., 1974) and the ice sheets of Greenland (Weiss et al., 1971). Biologically mediated volatilization processes can account for 30–50% of total Se emitted annually.

2.1.2 Anthropogenic activities

Globally, the activities of mankind such as emission from industrial activities has come forth as main source of atmospheric distribution of selenium and these are on higher side as compared to natural emission of selenium. At global scale, Se emission through anthropogenic sources to environment account for about 50-65 % of total environment (Mosher and Duce, 1987). Anthropogenic activities are estimated to release around 88,000 tons of Se per year to environment on global scale (Fordyce, 2005). The major sources of air-borne selenium include combustion of lignite, hard and brown coals in electrical power plants and in residential as well as commercial business. Among the main Se releasing anthropogenic processes, 7,300 tons of Se is liberated per year through the process of coal combustion only (Yudovick and Ketris, 2006). Selenium contaminated air, also plays a crucial role in possible contamination of water bodies.

The major sources of Se contamination of the aquatic ecosystem including the ocean are: coal burning power plants (45%), non-ferrous metal smelters (28%), domestic waste water effluents (9%), and dumping of sewage sludge (4%). Water also contributes towards addition of Se in environment as a result of irrigation through Se-rich water (Reilly, 2006). The contamination of surface water with drainage from irrigation water in soil of Punjab (Dhillon and Dhillon, 2003b; Bajaj et al., 2011) and San Joaquin valley of California (USA) (Wu, 2004; Luoma and Presser, 2009) are examples of Se addition in environment through anthropogenic activities.

2.1.3 Selenium in soil

Selenium content is found to be highly variable in soil across the world with concentrations ranging from below detection limits to 12.5 g/kg. Selenium content in soil is determined by number of factors such as Se content in soil native substrate, climate and age (Mayland et al., 1989). Apart from these factors, some other activities like amount of rainfall also plays considerable role in determining Se content of soil. The selenium content of soil varies from one country to other country, due to the fact that Se content mainly relies on concentration of this element in native substrate, which varies from region to region. The soils can be classified as seleniferous or non-seleniferous depending upon the Se level of non-accumulators (cultivated agricultural crops) grown on that soil. The soils containing 0.1–0.5 mg/kg of selenium (Ravikovitch and Margolin, 1957; Dhillon et al., 1992) are considered as seleniferous because the forages grown on such soils contain 4 mg Se/ kg, i.e., the maximum permissible level for animal consumption.

In most of soils, Se content varies in the range of 0.1 to 2.0 mg Se/kg, however the Se levels most frequently observed are found to be 0.2 and 0.4 mg Se/kg (McNeal and Balistrieri,

1989). Selenium content in soil varies from low to toxic ranges; low in the range from 0.03-0.08 mg/kg and toxic (seleniferous) levels can extend upto ≤ 1200 mg/kg (NAS,1976; Fordyce, 2005). Some parts of the world such as New Zealand, Denmark, Siberia, Finland and certain regions of Northeast to South-Central China are low in Se in their soils as well as crop systems (Dhillon and Dhillon, 2003a). The southern and western regions of Australia along with NewZealand are also found to be Se-deficient (Welsh et al., 1981). In India, particularly the soils of Haryana and Punjab are found to in toxic range (Dhillon and Dhillon, 1991). The European countries, particularly Wales and Ireland are registered with Se toxic profiles (Fleming, 1962). In India, total and water soluble Se in soils of Se affected regions of Haryana and Punjab were found to be lying in the range of 0.02 – 10 mg/kg (Singh and Kumar, 1976; Dhillon et al., 1992). In India, particularly in the Nawanshahr–Hoshiarpur region of Punjab, a large area of agricultural land (about 1000 hectares) is severely affected with high Se levels (Dhillon and Dhillon, 2003a). Furthur, studies carried out by Sharma et al. (2009), examined Se concentrations in soil and crop products (wheat husk, rice, maize and mustard), using neutron activation analysis, and found it be lying in the range of 2.7 - 6.5 mg /kg and 13 - 670 mg/ kg respectively, with significantly high Se content in these crop products. In the sub-Himalayan regions of West Bengal, the Se content of soils from the toxic pastures ranged from 1.45 to 2.25 mg/kg. Soils from the shales of North Dakota, USA, contain as much as 0.09 g Se/kg, while most non-seleniferous soils contain appreciably < 0.002g Se/kg (Trelease, 1945; Ermakov, 1992).

2.1.4 Selenium in food

The selenium content in soil, which varies from one place to another, has an important role in determining the Se content of food. The Se content of the food ingested depends on the

selenium content of soil native substrate (used for cultivating crops) and on residues (used for feeding animals) left over after harvesting. The Se content of among different foods is found to be lying in the range of 10-500 μg/kg. Cereals, legumes, cruciferous vegetables, fruits, meat, egg and dairy products are the main food groups serving selenium in diet (Mc Naughton and Marks, 2002)

Certain plants, termed as hyperaccumulators, accumulate Se at extremely high levels from the soil on which they were grown. Plants are divided into three types depending on the extent of Se accumulation; they include non-accumulators, selenium indicators and Se accumulators (Rosenfeld and Beath, 1964; Brown and Shrift, 1982; Dhillon and Dhillon, 2003a). Non-accumulator plants cannont grow on seleniferous soil and experience toxicity at tissue concentration upto 100 mg Se/g DW (Rosenfeld and Beath,1964; White et al., 2004), on the other hand Se indicators plants can grow on both Se and non-Se soil and can tolerate concentrations of approximately 1000 mg/kg (Rosenfeld and Beath, 1964; Rodriguez et al., 2005). Some of these rich sources of dietary Se, are listed in Table 1.

Cereals are the main staple food and dietary source of selenium across world, which are succeded by fruits, vegetables and other products. Cereals possess ten folds higher total Se content than meat. Wheat flour and bread are important sources of Se in diet schedule of United Kingdom (Barclay et al., 1995). Different strategies such as use of Se-rich fertilizers along with supplementation of animals with Se-rich feed/fodder have been practicised in areas low in Se content.

Table 2.1. Selenium content of some selected foods and food groups (Reilly, 1996)

	Se content (µg/g, fresh weight)
Food groups:	
Cereals, cereal products	0.01- 0.55
Meat, fish, eggs	0.01 - 0.36
Milk, dairy products	<0.001 - 0.17
Vegetables, fruit	<0.001- 0.022
Selected high-Se foods:	
Beef kidney	0.78 -1.45
Brazil nuts	0.85 - 0.53
Broccoli	<0.001 - 0.46
Crab	0.028 -1.26
Lobster roe	0.08 - 4.43
Mushrooms	0.01 -1.40

2.1.5 Selenium bioavailability and speciation in food

Bioavailability is defined as the quantity of nutrient ingested, which in turn is absorbable and utilizable through individual's diet (Van Campen and Glahn, 1999). Both inorganic and organic species of selenium could be easily absorbed by the body (Suzuki et al., 1998). Among inorganic forms, selenate and selenite are less easily absorbed and selenite is found to be more toxic than selenate (Magnuson et al., 1997) however the organic forms, (such as selenomethionine) are considered more bioavailable than the inorganic forms (selenites or selenates). Selenomethioneine is the predominant form of selenium reported to be present in diet (Swanson et al., 1991). Selenium is specifically incorporated into proteins and is responsible for their enzymatic activity (Sturchlerpierrat et al., 1995). On the other hand, Se in the form of SeMet is non-specifically incorporated into haemoglobin and plasma (Burk et al., 2001) and is retained in the tissues to larger extent than other species. Supplements mainly containing SeMet can maintain the expression of selenoenzymes during Se deficiency for much longer times than inorganic sources due to recycling of SeMet from protein stores (Combs, 1988). Apart from these there are other organoselenium methylated forms which are produced as a result of

detoxification (Pyrzynska, 1998) and are excreted. Another important product of selenium metabolism which is excreted via urine is trimethylselenonium ion (Levander, 1972). Bioavailability of selenium is affected both by the chemical form as well as other dietary components of Se. Blood is the main biological sample that is often used to indicate the selenium status. The five selenium containing proteins used as Se indicators in blood are tabulated in Table 2.2

Se absorption is not known to be under homeostatic control. The overall bioavailability of dietary selenium is not due to its absorption only, rather its conversion within tissue to its metabolically active forms such as GSH-Px and 5 deiodinases (Contempre et al., 1996).

Table 2.2. Biological markers used as indicator of selenium status in blood (Arthur, 1994)

S.No	Se-containing proteins	Location	Function
1	Plasma glutathione peroxidases (pGPx)	Plasma	Indicator of recent dietary selenium uptake
2	Selenoprotein P (SepP)	Plasma	
3	Albumin	Plasma	
4	Cellular glutathione peroxidases (cGPx)	Red blood cells	Reflects longer term selenium status, responding to dietary
5	Haemoglobin	Red blood cells	changes over time

Selenium exists in both in organic and inorganic chemical forms in foods and biological materials (Lobinski et al., 2000; Dumont et al., 2006). Selenium from plant forms is more bioavailable than from animal food stuffs (Combs, 1988; Bugel et al., 2002). SeMet is the the main dominant form that occurs in cereals, yeast, beans and mushrooms (Olson et al., 1970; Reilly, 1996; Bhatia at al., 2013). Food sources such as Brazil nuts and wheat that contain high

proportions of SeMet are considered to be highly bioavaliable (WHO, 1987; Ip and Lisk, 1994). Although, mushrooms are known to be potential selenium accumulators, yet bioavailbility in terms of selenium absorption is known to be relatively low (Fordyce, 2005). On the contrary, many recent reports have shown SeMet to be the dominant species present in mushrooms (Bhatia et al., 2013) with good bioavailability (Rayman et al., 2007).

2.2 Selenium intake: Global variation

The Se intakes in diet on individual basis was estimated to be lying in the range of 3-7000 μg/day (Rayman, 2002; WHO, 2004; Fordyce, 2005; Rayman, 2008).The seleniferous regions of China has been reported to have higher levels (>4990 μg/day) of dietary Se intake (Yang et al.,1983). The mean Se intakes for European countries were found to be lying in the typical range of < 50 μg/day per person, (Rayman, 2004; Rayman, 2005), which is found to be quite near or lesser than the recommended nutrient intake level the (Rayman, 2008; Flynn et al., 2009). Similarly, among Asian contries such as India, the Se intakes for men and women were estimated to be 632 μg/day and 475 μg/day respectively which are considered to be relatively high (Hira et al., 2003).

Amongst the countries recording low intake of Se, in United Kingdom, the intake for men and women were found to be 55 and 43 μg/day respectively. The intake is much higher in United States with the mean value for women and men intake to be 92.6 ± 1.57 and 133.5 ± 2.42 μg/day respectively (USARS, 2008).

2.2.1 Essentiality and toxicity of selenium

The term essential trace element is usually conferred to mention an element with an established or suspected requirement of < 1 mg /day (RDA, 1989). Selenium is a nutrient with

multiple metabolic fates, determined in part by dosage levels, thus it can be associated with impaired performance at both extremes of intake, so a balance between the two extremities is the utmost need of a biological system. Selenium exists as a two sided coin with both toxic and essential profiles; this particular feature makes the element immensely important from health point of view for animals and humans. The recommended dietary allowance (RDA) for Se is 55 µg per day for adult [IOM, 2000], whereas WHO states that the average intake level needed for required activities in healthy individuals is 40 µg per day (Combs, 2001). As per the recommendations of various European associations on nutrition, the minimum Se concentration require to combat diseases is found to be 30–70 µg per day for adult and the upper tolerable limit is fixed at 400 µg/day (Hurst et al., 2014). The Se requirement for most farm animals is between 0.1 and 0.3 mg/kg of feed (Mayland, 1994).

2.3 Health implications of selenium

The margin between deficiency, essentiality, and toxicity of selenium is found to be very narrow. Intake of food containing < 0.1 µg Se/day leads to deficiency, while consumption of food containing < 1µg Se/day leads to toxicity. The effect of selenium is concentration dependent and ranges from essentiality to anti-oxidant at nM- µM concentrations and further on to potentially prooxidant at concentrations above what is required for maximal selenoprotein synthesis (Vinceti et al., 2001). Furthur intake can lead to accumulation of intracellular thiols leading to toxic effects.

2.3.1 Toxic effects of selenium

The toxic nature of Se became known even much before it was discovered. Primarily Se was mainly considered as environmental toxicant till it was discovered as an essential nutrient

for animal and human health (Schwarz and Foltz, 1957). Marco Polo, while traveling in the remote parts of Western China and Eastern Turkestan in 13th century was the first to come in contact with probable Se poisoning. In later period, the occurrence of alkali disease was reported in Western United States (Rosenfield and Beath, 1964). It was attributed to strong relationship between the consumption of locally available forages and disease symptoms in horses. The symptoms observed in animals were impaired growth, poor reproduction as well as cracked and bleeding hoofs, poor growth and reproduction along with few cases of mortality. Another environmental concern regarding selenium came under notice in 1980's, wherein excess Se caused malformations of bird embryos, death of aquatic birds and poisoning of fish in Kesterson wild life refuge and reservoir in California. Draize and Beath (1935) also explained the symptoms appearing due to acute selenium toxicity such as loss of their control over voluntary muscles as a result of Se toxicosis.

There are no documented instances of naturally occurring Se causing damage to agricultural plants in the field (NAS–NRC, 1976). The Se-toxicosis in the form of snow-white chlorosis along with pink coloration was recorded for the first time, in lower region of leaves and sheath of wheat, under field conditions in the seleniferous areas of Punjab, India (Dhillon and Dhillon, 1991)

2.3.1.1 Toxic profile of selenium in organisms

Daily dosage of 900-1600 μg/person can cause adverse health effects and it is reported that selenosis occurs at intake of 3200-5000 μg/person (Combs, 2001; Whanger, 2004; Letavayova et al., 2006). Selenosis (Se poisoning) can be acute or chronic. Acute toxicity is mainly related to possible occupational exposure of people working on microelectronics, xerox,

and semiconductors; showing symptoms mainly related to respiratory systems like dypsnea, bronchitis, and pneumonia and sometimes leading to gastrointestinal and cardiovascular effects. Chronic effects are mainly due to high concentration of Se in food and water bodies, leading to hair loss, nail deforming and discoloration of skin and teeth.

The inorganic forms of selenium are recognized to be more acutely toxic than the organic forms. The organically bound Se compounds such as selenoaminoacids and volatile forms (dimethyldiselenide and dimethylselenide) are relatively less toxic as compared to other species. The order of selenium toxicity from several sources was found to be in the order of: selenate > selenite > selenide > metallic selenium (Franke and Painter, 1938). Some selected selenium compounds with their toxicity are listed in the Table 2.3.

The seleniferous regions across the world such as, Canada, Ireland, Western USA, India and China were found to be adversely effected by excess of selenium (Hira et al., 2003; Navorro-Alarcon and Cabrera-Vique, 2008). In China, outbreak of illness in terms of loss of hair and nails was noted which was later diagnosed to be severe selenosis due to high selenium content in soil (Yang et al., 1983). Similarly in India, in parts of Punjab state (Northwestern region of India); observations on human population has also shown signs of selenium toxicity with chronic selenosis mainly through consumption of Se hyperaccmulated crop products produced in Se-rich agricultural fields (Hira et al., 2003). Animals, particularly cattle, were also affected severely with Se toxicity due to high content of Se in fodder containing upto 5–160 mg Se/kg in the respective areas (Dhillon and Dhillon, 1991). Dey et al. (1999) studied the wild leopard cat (*Felis bengalensis*), civet cat (*Vivera zebith*), flying squirrel (*Petaurista magnificus*) and leopard (*Panthera pardis*) from different locations in Northeastern India. The content of Se was found to be high in the bones and in the hair it exceeded (1.5–2.6 times) toxic limits in all four species.

Table 2.3. Selected selenium compounds and their toxicity (Bueno et al., 2007)

Compound	Formula	Lethal dose LD-50*	Ref.
Dimethylselenide (-II)	$(CH_3)_2Se$	1600 mg/kg (Int.)	Al-Bayati et al., 1992
Hydrogen selenide (-II)	H_2Se	0.02 mg/L(Resp.)	Wilber, 1980
Trimethylselenonium (-II)	$(CH_3)_3Se^+$	49 mg/kg (Int.)	Wilber, 1980
Selenocystine (-I)	$[HO_2 CCH (NH_2) CH_2Se]_2$	35.8 mg/kg (Or.)	Sayato et al., 1993
Selenomethionine (-II)	$CH_3Se(CH_2)_2CH(NH_2)CO_2H$	4.3 mg/kg (Int.)	Wilber, 1980
Selenite (+IV)	SeO_3^{2-}	3.5 mg/kg (Int.)	WHO, 1987
Selenate (+VI)	SeO_4^{2-}	5.8 mg/kg (Int.)	WHO, 1987

*Lethal dose obtained on mice or rats by intraperitoneal (Int.), oral (Or.), or respiratory (Resp.) absorption

2.3.2 Selenium: Its essentiality to human health

Although selenium is toxic in large doses, yet it is an essential micronutrient for health. Selenium is a vital constituent of various metabolic pathways such as thyroid metabolism, antioxidant defense and immune functions. Selenium is an active integral part of glutathione peroxidase, which protects the cells from oxidative damage. Hydrogen peroxide and lipid hydroperoxides produced by lipooxygenase and cyclo-oxygenase pathways are effectively reduced by the antioxidant activities of glutathione peroxidase (Spallholz et al., 1990). The biological functions of selenium are mainly exerted through selenoproteins. Out of all, only 25 mammalian selenoproteins so far have been recognized on the basis of functional characterization. Most of the selenoproteins carry out redox activity as enzymatic functions

possibly due to presence of selenocysteine. Regulation of redox signaling, elimination of damaging/signaling peroxides, biosynthesis of dNTPs for DNA, selenium transport and storage, reduction of oxidized proteins as well as membranes, thyroid hormone metabolism and protein folding are the main cellular processes established so far that requires involvement of selenoproteins. Selenium is also proved to play a vital role in cancer prevention (Clark et al., 1996; Yu et al., 1997). Thioredoxin reductases, which are selenoenzymes, have important role in rejuvenation of antioxidant systems and to sustain intracellular redox state (Allan et al., 1999). The thyroid gland has highest concentration of Se as compared to any other organ. (Kohrle, 1999). Selenium facilitates the synthesis of thyroid hormone (T3) from thyroxine (T4) through iodothyronine deiodinases (Beckett et al., 1987). Supplementation of iodine and selenium is advised in particular (deficient in iodine and selenium) regions of central Africa where deficiency diseases such as goitre and myxedematous cretinism are more prevalent (Vanderpas et al., 1993).

The immune response of body towards infections is influenced by the role of selenium in various aspects. For example, deficiency of selenium reduces immunocompetence that leads to impairment of macrophages, neutrophils and leukocyte activity (Boyne and Arthur, 1986; Spallholz et al., 1990). Kiremidjian- Schumacher et al. (1994), reported that supplementation of selenium of Se deficient population stimulates immune system and enhances the proliferation of active T cells, natural killer cells and lymphocyte activity. The beneficial effects of synthetic organoselenium compounds and selenoproteins have been studied for their role as cytokine inducers, enzyme inhibitors, antioxidants and antitumour agents (Ganther, 1999; Mugesh et al., 2001). Supplementation of selenium has been reported to improve the conditions of patients suffering from inflammatory conditions such as septic shock, rheumatoid arthiritis, autoimmune

thyrioditis, allergic asthma and pancreatitis (Gartner and Angstwurm, 1999; Rayman, 2000; Gartner and Gasnier, 2003). Selenium exerted its anti-inflammatory response in activated macrophages through the enhanced production of 15d-PGJ_2 (15-deoxy-Δ 12, 14-prostaglandin J_2) in a cyclooxygenase (COX-1) dependent process (Vunta et al., 2007). Furthur, administration of selenium to Se-deficient macrophages leads to decline in the LPS-stimulated expression of important pro-inflammatory functions through cyclooxygenase-2 (COX-2) and tumor necrosis factor-a (TNF-a) (Vunta et al., 2008). Prabhu et al. (2002) and Gandhi et al. (2013), proposed the beneficial effect of dietary selenium supplementation in order to prevent the oxidative stress mediated inflammatory diseases.

2.3.2.1 Selenium-Effects of its deficiency in diet

A narrow range exists between deficiency of Se (< 40 μg/day) and its toxic implications (>400 μg/day). Se deficiency is more widespread than selenosis. The primary evidences of Se deficiency were the occurrence of two specific diseases i.e. Keshan and Kashin-Beck disease in some low Se areas of China. Keshan disease is a cardiovascular disease that involves cardiac enlargement due to damage of cardiac function mostly affecting young women and children (Reilly, 1996). On the other hand, Kashin-Beck disease, an endemic chronic degenerative ostreoarthropathy results in shortened toes and fingers, enlarged joints and dwarfism in rare cases (Reilly, 1996). The Keshan disease is likely due to number of factors in combination such as vitamin E deficiency and prevelance of coxsackie B virus along with deficiency of Se (Yang et al., 1994; Levander and Beck, 1999; Liu et al., 2002). Supplementation of individuals with Se tablets of sodium selenate has been found to be successful in checking the progress of Keshan disease (Yang, 2006). Similarly, the etiology of Kashin-Beck disease is also complex linking deficiency of Se, vitamin E and iodine (Neve, 1999). Health implications associated with

oxidative stress can be alleviated with Se and that includes asthma (Shaheen et al., 2001; Jahnova et al., 2002), osteoarthritis (Kurz et al., 2002), muscular dystrophy (Kurihara et al., 2000), diabetes (Kowluru et al., 2001), systemic inflammatory response syndrome (Angstwurm et al., 1999), cystic fibrosis (Kauf et al., 1994), kwashiorkor (Ashour et al., 1999) and arthritis (Peretz et al., 2001). Selenium deficiency has also been associated to several clinical manifestations like decrease in immune and thyroid functioning, various neurologic conditions, such as Alzheimer's and Parkinson's disease, increased risk of cancer and infection and male infertility (Rayman, 2000).

Several studies have confirmed that individuals with low plasma Se have potential risk of cancer (Clark et al., 1984, 1993). Certain human trails also revealed an inverse relationship between dietary selenium uptake and cancer risk in humans (Vogel and Mcpherson, 1989; Ghadirian et al., 2000; Diwadkar-Navsariwala and Diamond, 2004; Sabichi et al., 2006). In contrast, studies carried out in *in-vitro* and animal models indicate that selenium intakes at supra-nutritional levels can suppress generation of tumours (El-Bayoumy, 1991; Combs and Gray, 1998; Ip, 1998).

The biological activities of selenium, as a cancer-preventive agent, depend on its chemical forms. Sodium selenite and selenomethioneine are reported to inhibit cancer in many *in-vivo* conditions (Ip, 1986; Medina and Morrison 1988; El-Bayoumy, 1991; Combs, 1997). Selenomethioneine was found to be less effective than sodium selenite in terms of cancer inhibition (Ip and Hayes, 1989). However selenobetaine and Se-methylselenocysteine (the precursor for generating methylated forms) were found to be more efficient than selenite or selenomethioneine (1-3 ppm) against cancer prevention (Ip and Ganther, 1990, 1992; Ip et al., 1991). The probable reason for such observation is the incorporation of SeMet in tissue proteins

rather than entering the metabolic pathway thereby diverting its route for participation in anticancer pathway (Ganther, 2001). The degree of methylation is another important factor in cancer studies such as fully methylated form of selenium. For example, trimethylselenonium ion was ineffective against cancer as it gets completely excreted out of the body (Vadhanavikit et al., 1993).

2.3.2.2 Other health effects

It has been well documented that selenium is also important for neuronal functions in brain (Rayman, 2000). Genetic inactivation of glutathione peroxidase leads to brain ischaemia due to increased sensitivity towards neurotoxins. Similarly, genetic inactivation of selenoprotein P reduces brain Se content leading to movement disorders in animals (Schweizer et al., 2004). Deficiency of selenium is linked with increased virulence over a range of viral infections (Taylor, 1997). Schrauzer (1998) reported the use of selenite therapy against acute septicaemia, lymphoedema and viral haemorrhagic fever. Selenium deficiency is linked to HIV wherein it has also been observed to be related to viral load and mortality (Baum and Shor-Posner, 1998; Campa et al., 1999; Baeten et al., 2001). Glutathione peroxidase and thioredoxin reductase are required for protection of thyroid from H_2O_2 (Beckett and Arthur, 2005) and iodothronine deiodinase is needed for synthesis of active thyroid hormone. It has been documented by Rayman (2000), that various clinical manifestations such as cognitive impairment, depression, anxiety and hostility are associated with low Se levels. It has been observed that sperm nuclei selenoprotein and phospholipid Gpx 4 are required for sperm motility and maturation (Pfeifer et al., 2001; Maiorino and Ursini, 2002).

Selenium provokes a dose-dependent protection against intestinal inflammation, and even moderate Se deficiency, seems to have negative effects as seen in human populations (Steinbrenner et al., 2011; Krehl et al., 2012; Barrett et al., 2013). The innate immune response in mice infected with pathogenic bacteria *Listeria monocytogenes* and *Citrobacter rodentium* has been reported to be impaired by Se deficiency (Wang et al., 2009; Smith et al., 2011). Several selenoproteins have been shown or proposed to affect immune functions. Selenoprotein Sepp1, provided host protection during an infection with the parasite *Trypanosoma congolense* (Bosschaerts et al., 2008). Selenoprotein K is required for the oxidative burst of activated macrophages (Huang et al., 2012). Se-proteins such as thioredoxin reductases (TrxR), selenoprotein K, glutathione peroxidases (GPx), selenoprotein S (SelS) ,selenoprotein P (SepP) act as antioxidant enzymes, regulate redox-sensitive and inflammatory signaling pathways and are involved in maintaining endoplasmic reticulum (ER) homeostasis (Papp et al., 2007).

High selenium (serum) concentrations are however, linked to occurrence of diabetes along with higher fasting plasma glucose and increased glycosylated hemoglobin levels (Laclaustra et al., 2009; Stranges et al., 2010). Steinbrenner et al. (2011), indicated potential risk of diabetes, leading to adverse effects of selenium through interference of Se compounds with insulin-regulated molecular pathways (Phosphoinostide-3 kinase) which may underlie some of pro and anti-diabetic actions of Se. These studies inferred that in Se-administrated population, high plasma Se levels were related with hyperglycemia along with enhanced plasma level of lowdensity lipoproteins, triglycerides and cholesterol (Bleys et al., 2007; Lippman et al., 2009).

2.4 Biofortification of selenium

The idea of fortification of food with essential minerals at pre-harvest stage is a newly emerging and experimenting concept. The biofortification concept enlightens the potential of agricultural as well as nutrition science to deal with the persistent problem of micronutrient malnutrition. Through, biofortification, researchers are attempting to provide farmers with crop varieties that can effectively reduce nutritionally related health problems through natural diet of human population (Ximenez-Embun et al., 2004). Biofortification programme, propagated across the globe, is focusing dominantly on three micronutrients viz., iron, zinc and vitamin A, as majority of world's population is suffering from their deficiencies. The various parameters required to increase mineral yield in edible tissue includes, enhanced uptake of mineral by roots or leaves along with effective distribution from plant to edible tissues that too in a non-toxic form (Welch and Graham, 2005).

Therefore, a better knowledge of Se metabolism in higher plants is to deal with the persistent problem of Se deficiency and its delivery to human and livestock population. For the purpose of genetic biofortification and phytoremediation, the Se uptake and metabolism in plant can be fully exploited. A great deal of interest has been generated on Se uptake in higher plants due to emergence of concept involving high Se-crops for Se-fortification (Zhu et al., 2009).

Selenium can efficiently be delivered via the soil-plant- animal-human system (Rayman, 2000; Combs, 2001). Among the edible crops, the notable sources of Se are wheat, pulses and certain vegetable crops (Sharma et al., 2007; Cubadda et al., 2009). Se-rich wheat, maize and mustard grown on selenium rich soil in India, were found to be potential sources of selenium (Jaiswal et al., 2012a; Jaiswal et al., 2012b). Cruciferous edible crops such as broccoli contain low levels of Se (0.1-0.3 μg /g DW), but when cultivated in the presence of adequate Se, many

have got the capability to accumulate Se at much higher magnitudes than normal (Finley et al., 2000; Banuelos, 2002). Therefore, selenium enrichment of edible matrices such as mushrooms also generates a scope for production of functional foods with high economic values.

2.5 Selenium mobilization from agri-residues to mushrooms

Recently, the agro-industrial wastes like cassava bagasse, apple pomace, sugar cane bagasse, coffee pulp/husk and sugar beet pulp have been effectively utilized towards various bioprocess activities for their proper usage. The waste products such as agro-industrial residues on one hand helps in synthesis of value-added products and on other hand their utilization solves the problem of atmospheric pollution (due to burning) which otherwise is an environmental concern. Worldwide, many agricultural/plant residues are generated from Se-laden sites and have been effectively used for generating value-added products such as mushrooms. Se-rich *A.bisporus* grown on Elephant grass and Rye grass from Se-laden sites with selenium concentration of 0.8mg/kg were found to accumulate selenium concentration of 3.2 mg/kg (Lin et al., 2014). Each year, in Punjab region of India, tons of agricultural residues are being generated which are either burnt as a regular practice or used for composting in some cases. The agri-wastes generated in seleniferous region of Punjab, contain significant levels of selenium accumulated in plant parts, as reported in leaves and straw of wheat, rice and cereals (Dhillon and Dhillon, 2003; Sharma et al., 2009), and therefore have potential use as substrates for cultivation of selenium enriched mushrooms. Phytoremediation of Se-laden (contaminated) agricultural wastes can be used as valuable organic source for cultivation of Se-enriched mushrooms. Moreover, the spent waste left over may furthur be used for livestock supplementation, thereby removing excessive selenium in matrices. Mushroom cultivation on

Se-rich substrates from such sites not only removes excess Se, but also generates the scope of being recognized as cash -valued bio-based products with potential health beneficial effects. Thus, these agricultural residues can effectively be used as substrates for mushroom cultivation.

2.6 Mushrooms: Prospective dietary source of selenium

Since many decades, different matrices such as vegetables, cereals, fruits, seeds and mushrooms have been studied for antioxidant properties, for searching natural sources of antioxidants (Wong and Chye, 2009). Edible mushrooms are very popular food crops due to their potential health properties such as antibacterial, antiviral, antitumour activities and for their role in immunomodulating treatments (Wasser and Weis, 1999), as they possess high vitamin, mineral and protein content (Kalac and Svoboda, 2000; Matilla et al., 2001). Edible fungi have the abilty to transform inorganic selenium into organic Se with enhanced physiological activity and absorption rate (Bhatia et al., 2013). As mushrooms contain relatively high protein content, it is expected that they have the ability to imbibe high concentrations of selenium preferably into selenoaminoacids (selenocysteine and selenomethineine) and furthur on to selenoenzymes and selenoproteins. Thus, mushrooms definitely offer an ideal choice for selenium biofortification study.

The selenium content in mushrooms is highly variable and is generally found on higher side than most of vegetables (Rayman et al., 2008). The Se content in the popular edible fruiting bodies of mushrooms covered over twice the magnitude and ranged between <1–20 μgSe/g dw, with highest levels observed in the species of *Boletus* mushroom (Falandysz, 2008). Mushrooms that are known to accumulate selenium accumulate include *Boletus edulis* (Stijve and Cardinale, 1974; Byrne et al., 1976; Piepponen et al., 1984), *Lycoperdon perlatum* (Kosta et al., 1974) and

mushrooms from the *Tubiporales* (Stijve, 1977; Quinche, 1983), *Agariceace* (Stijve and Besson, 1976; Stijve,1977; Quinche,1979; Quinche, 1983) and *Albatrellus* species (Stijve et al., 1998) are also well known Se accumulators. Wild mushrooms species of mushrooms such as *Boletus edulis* and *Albatrellus pes-caprae* have been reported to contain selenium in the range of 20-70 and 200 mg Se/g (Falandysz, 2008). Edible mycelia and fruiting bodies of various mushrooms cultivated on Se-rich substrates (inorganic and organic) are also known to accumulate high amount of selenium ranging from 3.0 -1500 µg Se/ g DW (Gergely et al., 2006; Tham et al., 2009; Estrada et al., 2009; Turlo et al., 2010; Cremades et al., 2012; Savic et al., 2012; Maseko et al., 2013). The Se-rich *A*garicus *bisporus* (Champgnon Mushroom) grown on substrates supplemented with inorganic selenium effectively absorbed and accumulated this element in fruiting bodies (Piepponen et al., 1984; Elteren et al., 1998; Stefanka et al., 2001; Gergely et al., 2006). In case of fruiting bodies of normal *A. bisporus* selenium (selenomethionine) was not found to be bound with proteins; however the fruiting bodies of same species cultivated on Se-fortified substrates have been found to possess Se (selenomethionine) bound firmly to proteins along with some unidentified Se-moieties. However, most of the studies with Se-enriched mushrooms are through cultivation on substrates supplemented with exogenous supply of inorganic Se salts with fewer attempts made to explore the feasibility of cultivating mushrooms through use of natural Se-hyperaccumulated substrates.

Selenium speciation has already been reported by various researchers in different Se-enriched mushrooms with detection of selenocompounds such as SeMet (Ogra et al., 2004; Gergely et al., 2006), SeCyst (Yoshida et al., 2005) and MeSeCys (Gergely et al., 2006) Selenium was reported to be present in the form of selenocysteine (SeCyst), selenomethionine (SeMet), methylselenocysteine (MeSeCys), and inorganic selenium in *Lentinula edodes* and

Agaricus bisporus (Stefanka et al., 2001). Similary Se-rich fruiting bodies of *Pleurotus florida* were reported to contain SeMet (73%) as the dominant organic moiety along with presence of some unidentified selenocompounds (Bhatia et al., 2013). In the observations conducted on Se-rich *Lentinula edodes;* SeMet was the main compound detected and this compound was proposed to be bound to proteins of high molecular mass (> 40,000 kDa) protein (Ogra et al., 2004). The chemical forms of selenium found in Se-rich *Ganoderma lucidum* were dominated by selenoproteins (56–61%), followed by selenopolysaccharides (11–18%) and other selenocomponents (13.8–20.0%) along with a small fraction (0.067–0.22%) of nucleic acid (Zhao et al., 2004). Summary of various Se-moieties in wild grown and cultivated mushrooms is presented in Table 2.4.

Table 2.4. Summary of Se-species distribution in mushrooms (Bhatia et al., 2013)

Mushroom species	Origin	Extraction	Analytical technique[a]	Se species[b]	Ref.
Albatrellus pes-caprae *Boletus edulis*	Wild grown	Chemical; enzymatic	HPLC-NAA and AFS	Se (IV); SeMet; $SeCys_2$; unknown compounds	Slejkovec, Van Elteren, Woroniecka, Kroon, Falnoga and Byrne, 2000
Agaricus bisporus	Enriched	Chemical; enzymatic	HPLC-AFS	Se (IV); $SeCys_2$;	Stefanka, Ipolyi, Dernovics and Fodor, 2001
Agaricus bisporus	Enriched	Enzymatic	HPLC-HG-AFS	$SeCys_2$; Se (IV); SeMet	Dernovics, Stefanka and Fodor, 2002
Boletus edulis	Wild grown	Chemical	HPLC-ICP-MS	LMW (<1.35-20kDa) HMW (~50 kDa)	Wuilloud, Kannamkumarath and Caruso, 2004
Boletus edulis	Wild grown	Chemical; enzymatic	HPLC-ICP-MS	MeSeCys; unknown compounds	Wilburn, Vonderheide, Soman and Caruso, 2004
Lentinula edodes	Enriched	Chemical; enzymatic	HPLC-ESI-MS	SeMet	Ogra, Ishiwata, Encinar, Lobinski and Suzuki, 2004
Ganoderma lucidium	Enriched	Chemical	HPLC-AFS	SeMet	Zhao, Zhao, Zhao, Chen, Tong and HU, 2004
Macrolepiota procera; Lepista luscina; Boletus luridus	Wild grown	Chemical	HPLC-ICP-MS	SeMet; unknown compounds	Huerta, Sanchez and Sanz-Medel, 2005
Agaricus spp.	Enriched cultivated	Chemical; enzymatic	HPLC-ICP-MS	SeMet; unknown compounds	Diaz Huerta, Fernandez Sanchez and Sanz-Medel, 2006
Lentinula edodes	Enriched	Chemical; enzymatic	HPLC-ICP-MS	SeMet; Se (IV); unknown compounds	Yoshida, Sugihara, Inoue, Chihara, Kondo, Miyamoto et al., 2005
Agaricus bisporus *Lentinula edodes*	Enriched	Chemical; enzymatic	HPLC-ICP-MS	Se (IV); $SeCys_2$; MeSeCys; SeMet; unknown compounds	Gergely, Kubachka, Mounicou, Fodor and Caruso, 2006

[a] NAA neutron activation analysis; AFS atomic fluorescence spectrometry; HG hydride generation; ICP-MS inductively coupled plasma mass spectrometry; HPLC high performance liquid chromatography;ESI-MS electrospray ionization mass spectrometry.[b] Identified species, listed in decreasing order of concentration. LMW low molecular weight; HMW high molecular weight.

2.7 Lacunae

The review of literature although indicated extensive studies on Se uptake by edible and non-edible mushrooms cultivated on substrates supplemented with Se exogenously, there is limited evidence on Se uptake in mushrooms and biological properties of the Se fortified mushrooms. The earlier observations reported by various researchers also show that Se uptake and speciation studies were carried out only in mushroom cultivated on substrates exogenously enriched with Se through Se supplementation in growth or aqueous medium. To the best of our knowledge, there is no study reported till-date on accumulation and speciation of Se in mushrooms grown on agri-residues with Se hyper-accumulated through natural processes. With the growing importance on enhancing the bioavailability of Se, edible crops that can accumulate selenium are being used as natural sources of Se neutraceuticals for both animals and humans in areas of Se-deficiency. Keeping in view, the limited studies on this aspect especially from natural substrate rich in selenium and the anticipated benefits facilitating re-use of waste agri-residues, the doctoral work had following objectives.

- To study the uptake and assimilation of selenium by edible mushrooms cultivated on Se-rich agricultural residues.
- To characterize Se fortified mushrooms for anti-oxidant and bio-active properties.

3.0 Material and Methods

3.1 Sample collection

The selenium rich post-harvest agricultural residues of wheat and paddy crops collected from the village of Jainpur 31°13' N, 76°21' E , Nawanshahr- Hoshiarpur region, Punjab, India), were used as substrates for cultivation of Se-enriched mushrooms. Similarly, non-seleniferous (control) agricultural residues collected from Patiala 30°35' N, 76°37'E, (Punjab, India) were used for cultivation of control mushrooms.

3.2 Mushroom cultivation

3.2.1 Culture collection

The strains of *Agaricus bisporus* (U-3), *Volvariella volvacea* (Vv-4) were procured from Punjab Agricultural University (PAU), Ludhiana (Punjab, India). The strains of five *Pleurotus* sp. viz *Pleurotus florida* (DMRP-136), *Pleurotus djamor* (DMRP-205), *Pleurotus sajorkaju* (DMRP-112), *Pleurotus ostreatus* (DMRP-2), *Pleurotus citrinopileatus* (DMRP-10) were procured from National Research Centre of Mushroom (NRCM), Solan (Himachal Pradesh, India). The fungal cultures were sub-cultured on Potato dextrose agar (PDA) medium and were stored at 4ºC till use.

3.2.2 Spawn preparation

Selenium rich wheat grains obtained from the seleniferous sites were washed and boiled (20 min) till they get softened. Boiled wheat was thoroughly mixed with 2% calcium sulphate and 0.5% calcium carbonate to absorb the excess moisture. The resultant mixture was filled in

glass bottles (approximately 500 gm/bag) and was inoculated with the desired culture in the form of discs (5mm). The inoculated bottles were incubated at 25°C for 7 days until there was visible growth of dense mycelium (completely run spawn). Similar procedure was followed for preparation of spawn for control mushroom by using non-Se wheat grains.

3.2.3 Spawning of substrates

3.2.3.1 Spawning of *Pleurotus* species

Prior to spawning, selenium rich wheat straw was disinfected by dipping in water containing 1.5% formalin. The treated straw was then inoculated with spawn (approx. 3.0 %) and was filled in plastic bags (3-4 kg/bag). The spawned bags were then incubated in growth chamber at 25 ± 3°C for approximately 15 days in suspended position till entire straw got tightly bound by mycelia (spawn run) with moisture content maintained at 70%. The plastic layers of completely colonized bags were torn off and were left suspended until fruiting. Similar conditions were followed to grow control mushrooms, with non-Se wheat straw as the substrate. Fruiting bodies of all *Pleurotus* species were collected after 4-5 days of emergence of fruitings, by cutting the base of the stipe with sterile surgical blade. The fruiting bodies were weighed and dried at 40ºC for near complete dehydration.

3.2.3.2 Spawning of *Agaricus bisporus*

The button mushrooms were cultivated on compost prepared from agricultural residues, following method outlined by Punjab Agricultural University, India (Khanna, 2003) with minor modifications. The spawn was prepared on wheat grains and used as seed for spawning following the procedure mentioned earlier (section 3.2.2).

a) Composting

The wheat straw was collected from seleniferous sites and was processed following the method mentioned earlier (Khanna, 2003). The straw was moistened with water to attain 70-75% moisture. Wheat bran and superphosphate were added during wetting process. The wetted straw was arranged in the form of a pile to allow its degradation with desirable microbial flora. After 24 h, the pile was turned to maintain homogeneous mixing. Similarly, seven turnings were given to the pile at different time intervals to ensure proper composting of straw. At the first, third, fifth and seventh turning, mollases, gypsum, furadyn and gama BHC were added respectively. After 26 days, the processed compost was ready to be spawned (Fig. 3.1)

Figure 3.1. (a) Unprocessed straw (b) processed compost

b) Spawning of compost

The compost prepared from the Se-rich straw was inoculated with the fungal spawn by thorough mixing method. After inoculation, the compost was filled in polypropylene bags (24" × 18") and kept in cultivation chamber (23 ± 25 ^{o}C) till complete running of fungal mycelia across compost. The colonized compost was then covered with casing soil and kept in cultivation

chamber (14-18 ^{o}C) till emergence of fruiting. After 15-20 day of casing treatment, fruiting bodies were harvested and preserved till use.

3.2.3.3 Spawning of *Vovariella volvacea*

The paddy straw mushroom was cultivated on Se-rich paddy straw collected from seleniferous sites. Se-rich paddy straw was arranged in bundles of 40 cm length and 10 cm width (Fig 3.2). Bundles were soaked in running water overnight. After wetting, the bundles were stacked in layers of four followed by spawning with the active culture. The spawned stacks were incubated at 35 ^{o}C till emergence of fruiting bodies. After 7-9 days of spawning, fruiting bodies were harvested and stored till use. Similar method was followed for cultivation of NSe (control) mushrooms.

Figure 3.2. Arrangement of paddy straw bundles

3.3 Mushroom yield and biological efficiency

Total yield was calculated as the fresh weight of mushrooms harvested upto third flush per 0.5 kg of dry substrate for *Pleurotus* and *Volvariella* and 5 kg for *A. bisporus* used for its

cultivation. Biological efficiency (BE) was determined by the ratio of fresh weight (g) of mushrooms to dry weight (g) of substrate and expressed as percentage (Chang et al., 1981).

[Biological efficiency % = Fresh weight (g) of mushrooms harvested / Dry weight (g) of substrate x100]

3.4 Estimation of total selenium

Fresh harvested mushrooms were air dried at room temperature and powdered using pestle and mortar. Selenium content was analyzed (Levesque and Vendette, 1970) in Se-rich and control straw belonging to wheat and paddy as well as in dried fruiting bodies of all mushroom species (control and experimental) from the first flush using florescence spectrometer (Perkin – Elmer LS45, USA) after a triacid (nitric acid+ perchloric acid+ hydrochloric acid) digestion followed by reaction with 2,3-diaminonaphthalene (DAN). In brief, 100 mg portion of the sample (fruiting body/straw) was weighed accurately in an acid washed kjeldahl flask containing 5 ml concentrated nitric acid. After 30 min at room temperature, 2 ml of perchloric acid (72%) was added and kept on kjeldahl digestion unit for 20 min until emission of $HClO_4$ fumes and condensation of acid in the neck of the flask. The process of digestion was considered to be complete when the condensation ring reached the top of the neck. The mixture was then allowed to cool to room temperature. To facilitate the reduction of selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}), 2 ml of 1.0 N HCI was added and the flask was placed in the water bath (100 ^oC) for 15 min. The digest was quantitatively transferred to graduated tubes (50 ml), washed and made to volume (25 ml) with 0.1N HCl. 1 ml of the digest was added to 200 µl of 1:1 formic acid and 200 µl of stabilizing solution (A 0.04 M solution of Na_2EDTA which contained 10% hydroxylamine hydrochloride ($NH_2OH.HCl$). The digest was titrated to pH 1.8 with 4.0 N NH_4OH and placed

in a water bath at 50 ºC for 10 min. 2 ml DAN (0.1% in 0.1 M HCl) was added to the reaction mixture, shaken thoroughly (20 sec) and kept in water bath at 50 ºC for 30 min. After cooling to room temperature, 5 ml of cyclohexane were added, and the contents were vigorously mixed, and allowed to separate. The organic phase (3 ml) was separated and assayed florimetrically at an excitation wavelength of 360 nm and emission wavelength of 520 nm. Se quantification in each sample was carried out by relative method using emission spectrum of NIST certified Se ICP standard solution (SRM-1349).

3.4.1 Selenium estimation using ICP-MS

Total selenium content in the cultivation substrates (*i.e.*, wheat grain and straw) and in fruiting bodies of *Pleurotus florida* was measured using ICP-MS with the support of ISS Rome, Italy. Microwave digestion was performed by means of a Milestone Ethos Pro labstation (FKV, Italy) with quartz closed vessels, equipped with an infrared temperature control system. Microwave irradiation was performed with temperature control and automatic continuous adjustment of power output, with the programme as follows: 37 min ramp to 180° C and 15 min at 180° C. Approximately 0.3 g of sample were submitted in triplicate to 1 h pre-mineralization with 3 ml HNO_3 and then added with 1.5 ml of H_2O_2. Total Se measurements were carried out in the Dynamic Reaction Cell (DRC) mode using rhodium as internal standard. The certified reference material NIST Durum wheat flour 8436 was included in each analytical batch for quality control. The accuracy of total Se determinations as assessed through NIST 8436 analysis was satisfactory wherein the certified value was 1.1 ± 0.2 µg Se/g dw and a trueness of 91% and precision of 3% were obtained. Total Se liberated by gastrointestinal digestion was also determined by ICP-MS. Aliquots of extracts (2 ml) were added with 0.5 ml HNO_3 and 0.5 ml H_2O_2 and submitted to the same digestion procedure as above.

Studies on *Pleurotus florida* was furthur extended to understand the species profile of Se-rich fruiting bodies. Se species liberated by simulated gastrointestinal digestion were characterised by size exclusion HPLC-ICP-MS and anion exchange HPLC-ICP-MS. Se species in extracts were identified by retention time matching with the standard substances spiked to the sample extracts. Quantitative calculations were based on peak areas. Measurements were carried out in standard mode with the operating conditions listed in Table 3.1.

3.5 Selenium bioaccessibility in mushrooms extracts

For selected samples viz. *Pleurotus florida, in-vitro* simulated gastrointestinal digestion was carried out to understand the extent of bioaccesibility of Se. The analysis was carried out with analytical support of ISS, Rome (Bhatia et al., 2013).

The *in-vitro* enzymolysis procedure simulating human gastro-intestinal digestion was carried out in triplicate. In parallel, procedural blanks were run to check the presence of Se in the reagents. Mushroom samples (0.5 g) were incubated with 5 ml of gastric juice (1% w/v pepsin in 0.15 M NaCl, adjusted to pH = 2 with HCl 37% v/v). After 1 min of vigorous shaking for initial degassing, the samples were placed in a mixing water bath (GFL 1083, Burgwedel, Germany) at 37 °C for 4 h. Samples were then adjusted to pH 6.8 with 2 M NaHCO. After adding 5 ml of intestinal juice (3% w/v pancreatin, 1.5% w/v amylase, 1% w/v bile salts in 0.15 M NaCl), samples were vigorously shaken for 1 min, degassed and further incubated for 4 h at 37 °C under gentle shaking. Samples were then centrifuged at 8000g and 4 °C for 15 min, the supernatant was collected, filtered through 0.45 μm membranes, divided into aliquots and stored at -80 °C until analysis for Se content.

Table 3.1. Instrumental operational conditions

ICP-MS Settings	
RF power	1.4 Kw
Nebulizer gas flow rate, Ar	1.04 L/min
Lens voltage	9.0 V
DRC gas flow rate, CH_4	0.7 ml/min
Rejection parameter q (RPq)	0.45
Analytical masses	^{80}Se (total Se/DRC mode), ^{77}Se, ^{82}Se (Se speciation/ standard mode)
Chromatographic Conditions	
Size exclusion chromatography	
Column	Superdex 75 10/300 GL (GE Healthcare)
Mobile phase	Ammonium acetate 100 mM, pH 7.5
Flow rate	0.5 ml/min
Injection volume	75 µl
Isocratic elution	0-60 min
Temperature	23 ºC
Anion exchange chromatography	
Column	Hamilton PRP-X100 (250 mm × 4.1 mm, 5 µm equipped with guard column)
Mobile phase	(A) Acetic acid 20 mM, triethylamine 10 mM (B) Acetic acid 200 mM, triethylamine 100 mM
Flow rate	1 ml/min
Injection volume	100 µl
Gradient elution	0-5 min: 100% A; 5-30 min: 0-100% B; 30-40 min: 100% B; 40-41 min: 0-100% A; 41-45 min: 100% A
Temperature	23 ºC

Source: Bhatia et al. (2013)

3.6 Elemental composition of mushrooms

3.6.1 CHN analysis

Carbon, hydrogen and nitrogen content (%) was determined in powdered sample of mushroom by using CHN analyzer (Flash EA 1112 series).

3.6.2 Elemental analysis

Powdered samples of mushrooms (100mg) were subjected to triacid digestion (HNO_3 : $HCLO_4$: HCl in ratio of 2:2:1) in microwave digestor (MARS 6, USA) with the programme setting as follows: 15 min ramp to 200 °C; 30 min at 200 °C. After cooling the digested fraction was filtered through Whatman no. 42 filter paper to 50 ml volumetric flask and was made to volume with double distilled water. A blank was also prepared using similar experimental procedure. Aliquots of digested samples were aspirated to atomic absorption spectrometer (AAS; GBC932AA, Australia) for determination of (Fe, Zn, Mg, Ca and Cu) elemental content.

Total protein content in the mushrooms was determined by the method of Lowry et al. (1951).

3.7 Determination of antioxidant properties

3.7.1 Preparation of extracts

Prior to use, the fruiting bodies were milled until a fine powder was obtained. One gram of dried samples were extracted by stirring with 10 ml of 50% methanol for 3 h, at room temperature using probe ultrasonicator, and filtering through Whatman # 1 paper. The filtrates were concentrated with a rotary vacuum evaporator at 40 ^{o}C. The resultant extracts were stored at 4 ^{o}C until use.

3.7.2 Total phenol content

Total phenolic content was analysed using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) using gallic acid as standard, with some modifications.

Briefly, in 0.1 ml of sample was added in 0.1 ml of Folin and Ciocalteu's phenol reagent. The mixture was incubated at room temperature for 3 min followed by addition of 0.1 ml of saturated Na_2CO_3 solution. The final volume was made up to 1 ml with distilled water. The reaction mixture was incubated for 90 min in dark condition. The absorbance was measured at 765 nm using UV-visible spectrometer (Hitachi – U2900). Gallic acid at concentrations of 0.05-0.10 mg/ml was used to make standard curve. The mean values were expressed as mg of Gallic acid equivalents (GAE) per gram of extract.

3.7.3 Lipid peroxidation by TBARS assay

Lipid peroxides were extracted by grinding the sample with 3 ml of 5% (w/v) metaphosphoric acid and 100 ml of 2% (w/v) butyl hydroxytoluene (in ethanol). Homogenates were filtered and centrifuged at 15000 g for 20 min. The chromogen was formed by mixing 500 µl of supernatant, 50 µl of 0.2% (w/v) butyl hydroxytoluene (BHT), 250 µl of 1% (w/v) Thiobarbituric acid (TBA) (in 50 mm NaOH), and 250 µl of 25% (v/v) HCl, and by incubating the reaction mixtures at 95°C for 30 min (Minotti and Aust, 1987). A blank for all samples was prepared by replacing the sample with extraction medium, and controls for each sample were prepared by replacing TBA with 50 mm NaOH. The reaction was stopped by cooling the samples in an ice bath. For determination of thiobarbituric acid reactive substances (TBARS), the chromogen formed was extracted by adding 2 ml of 1-butanol, the tubes were vigorously shaken, the organic (upper) phase was separated by low speed centrifugation and assayed fluorimetrically (Perkin-Elmer-LS45) at an excitation wavelength of 532 nm and emission wavelength of 550 nm. Calibration curves were made using malondialdehyde (MDA;Sigma) in the range of 0.5-5.0 µM.

3.7.4 Total antioxidant activity

Total antioxidant activity of methanolic extracts of mushrooms was measured according to the method (phospho-molybdenum assay) outlined by Imran et al. (2011). Different concentrations of BHT (0.1 – 1.0 mg/ml) were prepared. 0.3 ml of each of the methanolic extracts was taken in test tube to which 10 ml of 0.6 M sulphuric acid, 10 ml of 28 mM sodium phosphate and 10 ml of 4 mM ammonium was added. 0.3 ml of methanol served as blank. All the tubes were incubated at 95 °C for 90 min and cooled to room temperature. The optical density was measured at 695 nm using UV-visible spectrometer (Hitachi - U 2900).

3.7.5 2,2 Diphenyl 1-picryl hydrazyl (DPPH) scavenging assay

The scavenging activity of the methanolic extracts from mushrooms on DPPH radicals was measured according to the method of Chu et al. (2000). An aliquot of 900 µl of 0.1 mM DPPH (Sigma) taken in methanol was added to a test tube with 100 µl of mushroom extract. Methanol was used as a control. The reaction mixture was mixed at room temperature and the absorbance was determined immediately by measuring at 520 nm using UV-visible spectrometer (Hitachi - U2900). The scavenging activity (%SA) of DPPH radicals was calculated using equation [%SA= (1-Abs in the presence of sample/Abs in the absence of sample) × 100]. Butylated hydroxyl anisole (BHA) (0.05- 0.50 mg /ml) was used as standard.

3.7.6 Metal chelating activity

The chelating activity of the mushroom extracts for ferrous ions was measured following the ferrozine method (Dinis et al., 1994). 50 µl of the extract was mixed with 50 µl of ferrous chloride ($FeCl_2$, 2 mM). After 5 min, the reaction was initiated by the addition of 5 mM ferrozine

(0.1 ml), and the total volume was adjusted to 3 ml with deionized water or ethanol. The mixture was then shaken vigorously and incubated at room temperature for 10 min. The absorbance of the mixture was determined at 562 nm. The metal chelating activity of the mushroom extracts was calculated as: % chelating activity = [($A_{negative} - A_{sample}$) / $A_{negative}$] ×100, where A is absorbance. EDTA was used as positive control while absence of extract of the mushroom was the negative control.

3.8 Determination of cytotoxicity of Se-extracts on lung cancer (A549) cell lines

One gm of freeze dried mushrooms (both Se and NSe) were extracted with different solvents in order of their increasing polarity (hexane, ethanol, methanol and water) in the ratio 1:10 (w/v). The extracts were vortexed and subsequently sonicated for 3 min. The mixture was centrifuged at 6000 rpm for 10 min. The resultant supernatant was evaporated to dryness at room temperature. The residue obtained was weighed and reconstituted in respective solvents and were stored at -20 °C till use.

The extracts from various solvent fractions were screened using MTT assay according to previously described method (Mosmann, 1983) on A549 cell line for their anti-tumour activity. The Human alveolar adenocarcinoma (A549) cell line was grown in a humified atmosphere (5% CO_2) at 37 ºC in Dulbecco's modified essential medium containing 10% FBS and 1% Pen-Strep (Pencillin-Streptomycin). For MTT assay, the adherent cells were detached by trypsinization from 25cm^2 flask on the first day. The viable cells were counted by using 0.5% Trypan Blue dye exclusion method and cell count was adjusted to 2 x 10^5 cells /ml. 50 µl of various extracts was added in triplicates in 96 well microtitre plate and air-dried overnight under aseptic conditions to let the solvent evaporate completely. Wells with solvent alone were kept as controls and dried in

similar way. The cells were then seeded using a concentration of 2 x 10^5 cells/ml (100 µl/well). The microtitre plate was incubated at 37 ºC, 5% CO_2 for 24 h. After incubation, the medium of each well was removed by aspiration and replaced with experimental bioactive residue (50 µl/well) and incubated for a period of 24 h. After this interval, 0.05 mg of MTT was added to each well and the plate was incubated for 4 h in CO_2 incubator. Subsequently after 4 h, the media was removed from each well and DMSO was added to all wells to dissolve the formazan crystals. Cell viability was determined by measuring the absorbance at 570nm using Biotek throughput reader (Power wave 34,USA). The inhibition ratio (I) was calculated according to the following equation: where A stands for absorbance of control; AB stands for absorbance of extract.

I (%) = [(Acontrol group – AB treated group)/Acontrol group] X 100

3.9 Preliminary investigations on selenoergothioneine like moiety in Se-fortified mushrooms

3.9.1 Extraction

One gram of freeze dried fruiting bodies were added to 20 ml of cold ethanolic extraction solution (10 mm DTT, 100 µm betaine, 100 µm MMI in 70% ethanol) and mixed by vortexing and subsequent sonication for 3 min. An ethanolic solution (4 ml) of sodium dodecyl sulfate (SDS) was added and mixed by mild shaking. The mixture was centrifuged at 4000 rpm for 15 min. 10 ml of the supernatant was evaporated using rotary vacuum evaporator to dryness and the residue was re-suspended in 10 ml of distilled water (pH 7.3) followed by centrifugation at 4000 rpm for 15 min. The resulting supernatant was further used for the analysis.

3.9.2 FTIR and LC-MS based analysis

The Se-fortified mushrooms were analysed by FTIR for presence of selenoergothioneine - like moiety. FTIR analysis was carried out for the extracts wherein infrared spectra were recorded in the range of 4000-500 cm on Magna FTIR-550 spectrophotometer using KBr pellets. Following FTIR, samples (extracts prepared for selenoergothioneine analysis) were analyzed by HPLC (Waters-Alliance 2795) equipped with X-terra C-18 column connected to Waters-Micromass Q-TOF mass spectrometer. The HPLC conditions were: injection volume 20 µl with isocratic mobile phase consisting of 30% acetonitrile and 1% acetic acid in water at a flow rate of 0.5ml/min. The MS conditions were: electrons spray ionization in positive ion mode, capillary voltage (3000-3250 V), source temperature (120 °C), and probe temperature (250 °C).

3.10 Statistical analysis

All estimations except CHN and mineral analysis were carried in triplicates and were expressed as mean ± standard deviation (SD) values. The comparison between Se and non-Se samples were drawn using student‘t’ test. The comparison between Se vs Se species was drawn using one way and two way ANOVA using Graph pad prism software 4.1.

4.0 Results

The present study was framed to cultivate Se-rich mushrooms on Se-rich agricultural residues belonging to wheat and paddy crops and to furthur study their uptake and accumulation profile with respect to control mushrooms (NSe). Similarly non- enriched mushrooms (as control) were also cultivated on normal agricultural residues to study the Se uptake and assimilation.

4.1 Mushroom cultivation on agricultural residues

Seven mushroom species belonging to different genera were cultivated on post agricultural residues belonging to both seleniferous and non-seleniferous (control) sites. Out of seven species, five belonged to genus *Pleurotus* viz., *P. florida*, *P. djamor*, *P. sajorkaju*, *P. citrinopileatus* and *P. ostreatus* (Fig. 4.1 - 4.3) and others belonged to genus *Agaricus* and *Volvariella* viz. *A. bisporus* and *V. volvacea*. All strains of *Pleurotus* and *Agaricus* mushrooms were cultivated on agricultural residues belonging to wheat crop; however *V. volvacea* was cultivated on agricultural residues belonging to paddy crop.

The average yield of all mushrooms belonging to both Se and NSe (control) on their respective substrates were recorded after a cycle of 3 flushes and is represented in Table 4.1 as total yield and biological efficiency % (BE). It was observed that selenium accumulation did not induced any significant difference in total yield and BE of mushrooms over non-Se (control). In general Se-rich mushrooms showed almost comparable yield when compared to their respective control. However when comparison was drawn among various Se-rich species, the order of increase was as follows *A. bisporus* > *Pleurotus spp.* > *V. volvacea*. The maximum yield (Fig.

4.4) was obtained with *A. bisporus* (986.4 ± 19.9 g/5 kg bag) cultivated on Se-rich compost followed by different species of *Pleurotus* mushroom (on Se-rich wheat straw), amongst which *P. sajorkaju* (400.4 ± 13.8 g/0.5kg bag) showed maximum followed by *P. ostreatus*, *P. citrinopileatus*, *P. djamor* and *P. florida* followed by *V. volvacea* (paddy straw mushroom) showing lowest yield (193.2 ± 23.6 g/ 0.5 kg bag). Biological efficiency (Fig. 4.4) was used to express the yield of the fresh fruiting bodies per g dry substrate. Se-rich mushrooms showed no significant difference in terms of BE over control mushrooms.

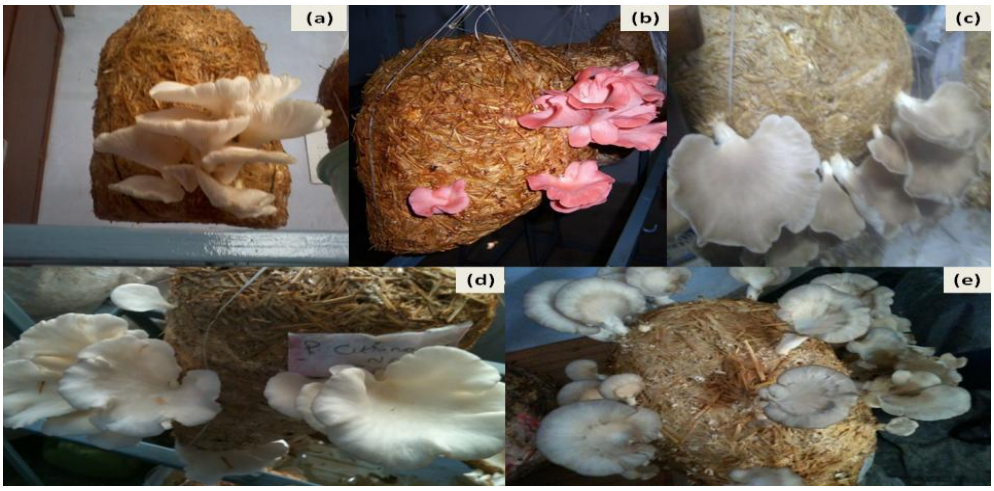


Figure 4.1. Fruiting of different *Pleurotus* species cultivated on Se-rich wheat straw; (a) *P. florida*, (b) *P. djamor*, (c) *P. citrinopileatus*, (d) *P. sajorkaju*, (e) *P. ostreatus*

Figure 4.2. Fruiting bodies of paddy straw mushroom (*V. volvacea*) cultivated on Se-rich paddy straw

Figure 4.3. Fruiting bodies of *A. bisporus* (button mushroom) cultivated on Se-rich compost

Among different genus, BE increased in the pattern different than total yield i.e. *Pleurotus* sp. followed by *A.bisporus* and *V. volvacea*. Among *Pleurotus* sp. maximum BE was shown by *P. sajorkaju* followed by *P. ostreatus* > *P. citrinopileatus* > *P. djamor* > *P. florida.*

Table 4.1. Total yield (g/kg dry substrate) and biological efficiency [BE (%)] in selenium enriched mushrooms and their respective controls (n=3)

Sample		Wheat straw						Paddy straw
		A. bisporus **(g/5kg)**	***P. florida*** **(g/0.5 kg)**	***P. sajorkaju*** **(g/0.5 kg)**	***P. ostreatus*** **(g/0.5 kg)**	***P. djamor*** **(g/0.5 kg)**	***P. citrinopileatus*** **(g/0.5 kg)**	***V. volvacea*** **(g/0.5 kg)**
Total yield	Se	986.4±19.9	324.9±7.20	400.4±13.8	391.7±14.6	350.0±30.1	374.0 ± 9.30	193.2 ± 23.6
	NSe	983.4±29.3	326.7±15.5	398.5±8.10	389.9±18.3	348.4±16.9	372.5 ± 11.0	195.1 ± 19.0
B.E (%)	Se	19.72±0.39	64.98±1.45	80.0 ± 2.76	78.34±2.93	70.0 ±6.00	74.80 ±1.86	19.32 ±2.36
	NSe	19.66±0.58	65.35±3.10	79.7 ±1.60	77.98±3.70	69.68±3.38	74.50±2.20	19.51 ±1.90
		ns	ns	ns	ns	ns	ns	ns

ns- non-significant

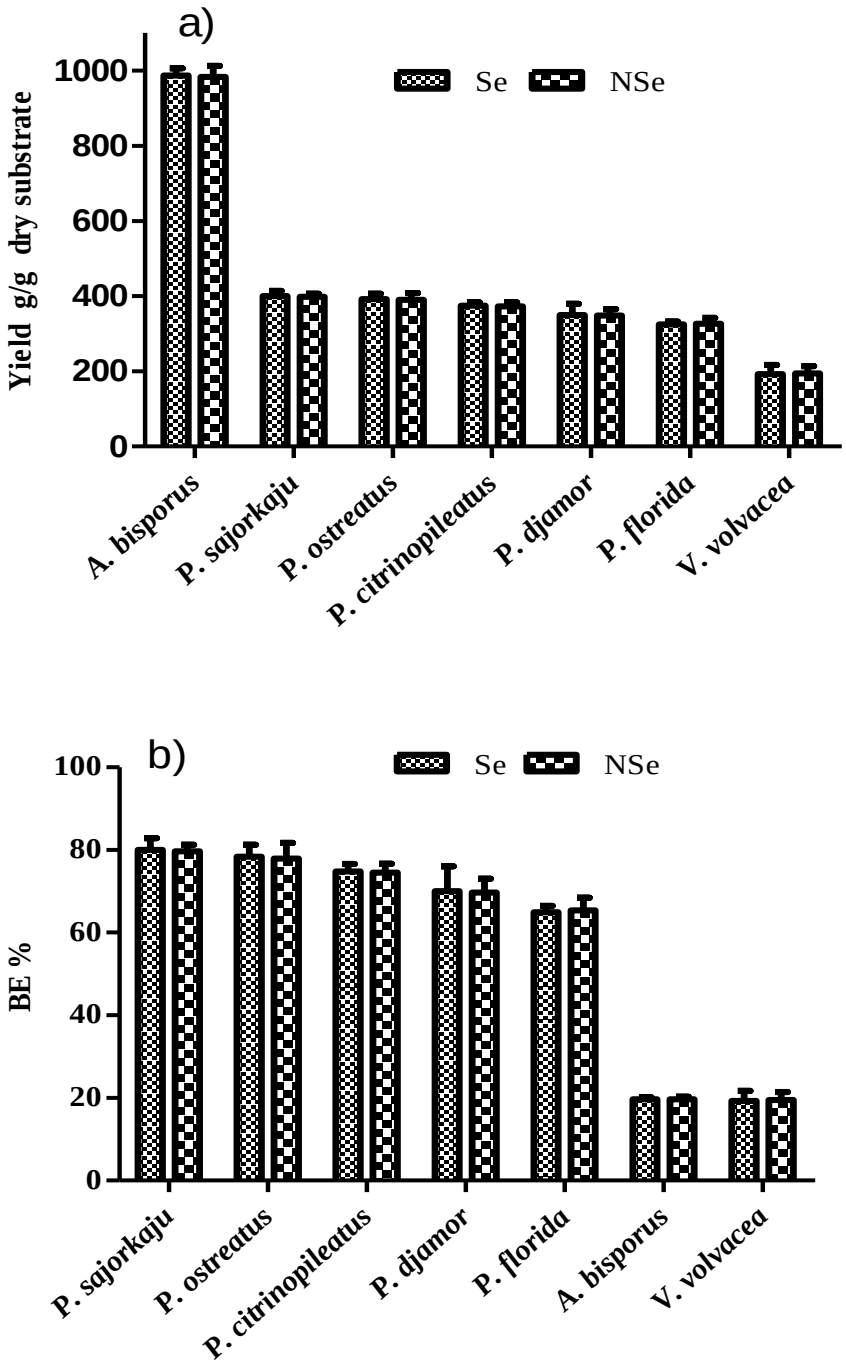


Figure 4.4. Total yield (a) and biological efficiency [BE (b)] in selenium enriched mushrooms and their respective controls (n=3)

4.2 Selenium uptake in mushrooms from substrates

4.2.1 Estimation of total selenium

Total selenium content in raw substrates (wheat and paddy) was analyzed using florescence spectroscopy and is presented in Table 4.2. The selenium content in Se-rich wheat (24.0 ± 0.2 µgSe/g DW) and paddy (29.7 ± 0.9 µgSe/g DW) straw was found to be significantly higher ($P < 0.001$) over their respective controls (1.9 ± 0.8 and 2.0 ± 0.6 µgSe/g DW). Se-rich paddy straw showed significantly higher ($P < 0.01$) selenium content than Se-rich wheat straw when comparison was drawn between different Se-rich substrates. Se-rich compost (processed Se-rich wheat straw) used for cultivation of *A. bisporus* was also found to contain significantly ($P < 0.001$) higher selenium content over non-Se compost. Similarly, level of selenium in Se-rich spent substrate (left after complete harvesting) bags belonging to both wheat and paddy were also analyzed to know the extent of Se removal from substrates and the residual amount of selenium left in bags, after being accumulated by mushrooms in three consective flushes. Se-rich spent wheat and paddy straw were found to contain 14.2 ± 0.12 and 15.8 ± 0.2 µgSe/g DW of selenium, which was found to be just half the amount of selenium present in raw substrates analyzed before cultivation.

The fruiting bodies (first flush) harvested from Se-rich substrates (wheat and paddy) were noted to contain significantly higher selenium content over control fruiting harvested from non-Se substrates (Table 4.3). Different genera of mushrooms accumulated varying concentration of selenium (Fig. 4.5) depending on the matrix of cultivation and selenium concentration in it. The fruiting bodies of *A. bisporus* harvested from Se-rich compost containing a total Se concentration (in dry weight) of 27.0 ± 0.3 µg Se/g DW were noted to accumulate Se

Table 4.2. Total selenium content in experiment (Se) and non-seleniferous (control) substrates collected from seleniferous and non-seleniferous sites (n=3)

Sample	Total Selenium(µg Se/g DW)				
	Wheat straw	**Paddy straw**	**Compost**	**Spent wheat straw**	**Spent Paddy straw**
Se	24.0 ± 0.2	29.7 ± 0.9	27.0±0.3	14.2 ± 0.12	15.8 ± 0.2
NSe	1.9 ±0.8 ***	2.0 ± 0.6 ***	1.5 ± 0.4 ***	BDL	BDL

(*: P<0.05; **: P<0.01; ***: P<0.001); BDL-below detection limit (< 0.5 µg Se/g)

upto 122.0 ± 1.8 µg Se/g DW as compared to control/ non-Se (NSe) mushroom (14.2 ± 0.7 µg Se/g DW) cultivated on control compost (1.5 ± 0.4 µg Se/g DW). Se-rich *A. bisporus* exhibited Se concentrations almost 8.5 times higher than control mushroom. Similarly, the fruiting bodies of all species of *Pleurotus* harvested from Se-rich wheat straw containig 24.0 ± 0.2 µg Se/g DW were noted to accumulate significantly higher ($P < 0.001$) Se as compared to control mushrooms grown on control wheat straw (1.9 ± 0.8 µg Se/g DW). Among five species of *Pleurotus*, *P. djamor* was found to accumulate high levels of Se as compared to control samples i.e. 145.4 ± 2.9 vs 5.0 ± 0.46 µg Se/g DW followed by *P. florida*, *P. ostreatus*, *P. sajorkaju* and *P. citrinopileatus*.

Table 4.3. Total selenium content in experimental (Se-enriched) mushrooms and their respective controls (n=3)

Sample	Total Selenium (µg Se/g DW)						
	A. bisporus	***P. florida***	***P. sajorkaju***	***P. ostreatus***	***P. djamor***	***P. citrinopileatus***	***V. volvacea***
Se	122.0±1.83	110.5±1.18	43.5±2.11	44.3 ±2.34	145.4±2.9	26.1 ± 2.80	35.0±1.16
NSe	14.24 ± 0.7 ***	3.29 ± 0.20 ***	5.2 ± 1.03 ***	3.42 ±0.07 ***	5.0 ±0.46 ***	2.97 ± 0.14 ***	6.0 ± 0.07 ***

(*** P<0.001)

The extent of Se accumulation in terms of fold increase is illustrated in Figure 4.5, wherein an increase of 8 - 33 folds has been observed to be exhibited by five strains of Se-rich oyster mushrooms over control. Similarly, the fruiting bodies of *V. volvacea* harvested from Se-rich paddy straw containing a total selenium concentration of 29.7 ± 0.9 µg Se/g DW were noted to accumulate significantly ($P < 0.001$) higher selenium (35.0 ± 1.16 µg Se/g DW) content as compared to control mushrooms (6.0 ± 0.07 µg Se/g DW) grown on control paddy straw (2.0 ± 0.6 µg Se/g DW). Se-rich *V. volvacea* fruiting bodies also exhibited Se concentration almost 6 times higher than control mushrooms. A statistical multiple comparisons (Tukey test) analysis (Fig. 4.6) was drawn between all Se-rich mushrooms in terms of selenium uptake (one-way ANOVA). A statistically significant difference was obtained at $P< 0.05$ between different species of Se-rich mushrooms. However, comparison between Se-rich *P. sajorkaju* vs *P. ostreatus* was found to be non-significant in terms of selenium uptake.

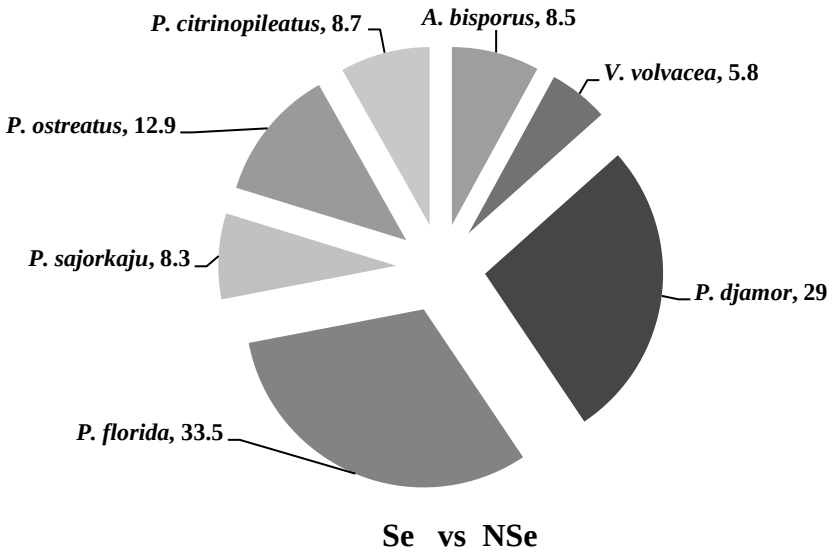
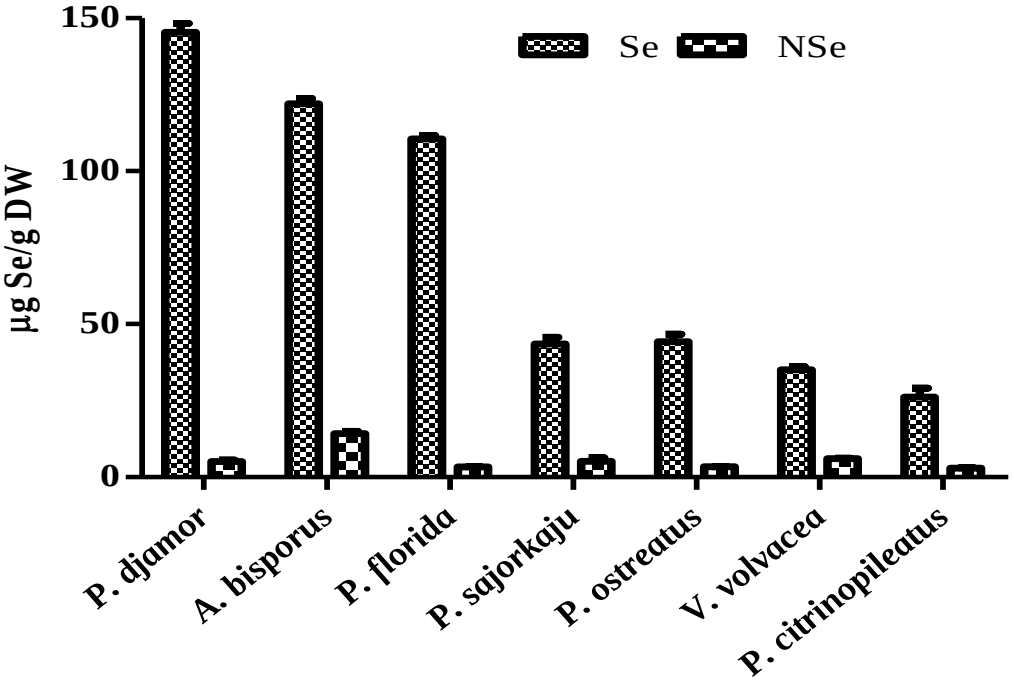


Figure 4.5. Selenium accumulation in Se-rich fruiting bodies in terms of fold increase over control (NSe)



One way ANOVA between various Se rich species	
(*** at P < 0.05)	
Multiple comparison analysis	
P < 0.001	P < 0.01
A.bisporus vs P. djamor	***P.citrinopileatus vs V.volvacea***
A.bisporus vs P. sajorkaju	***P.ostreatus vs V.volvacea***
A.bisporus vs P. florida	***P.sajorkaju vs V.volvacea***
A.bisporus vs P. citrinopileatus	
A.bisporus vs P. ostreatus	
A.bisporus vs V.volvacea	
P. djamor vs P. sajorkaju	
P. djamor vs P. florida	
P. djamor vs P. citrinopileatus	
P. djmaor vs P. ostreatus	
P. djamor vs V.volvacea	
P. florida vs P. citrinopileatus	
P. florida vs P. ostreatus	
P.florida vs V.volvacea	
P.citrinopileatus vs P. ostreatus	
P. sajorkaju vs P. florida	
P. sajorkaju vs P. citrinopileatus	

Figure 4.6. Total selenium content in experimental (Se-enriched) mushrooms and theirespective controls (n=3)

To study the intraspecies variation in terms of selenium accumulation, a comparison between different Se-rich mushrooms was drawn. Among *Pleurotus*, *P. djamor* showed the highest Se accumulation followed by *A. bisporus* and *V. volvacea*. *P. djamor* showed 1.2 and 4.2 times higher Se levels over *A. bisporus* and *V. volvacea* respectively, whereas *A. bisporus* showed almost 3.5 fold increase in Se levels over *V. volvacea* (Fig. 4.7).

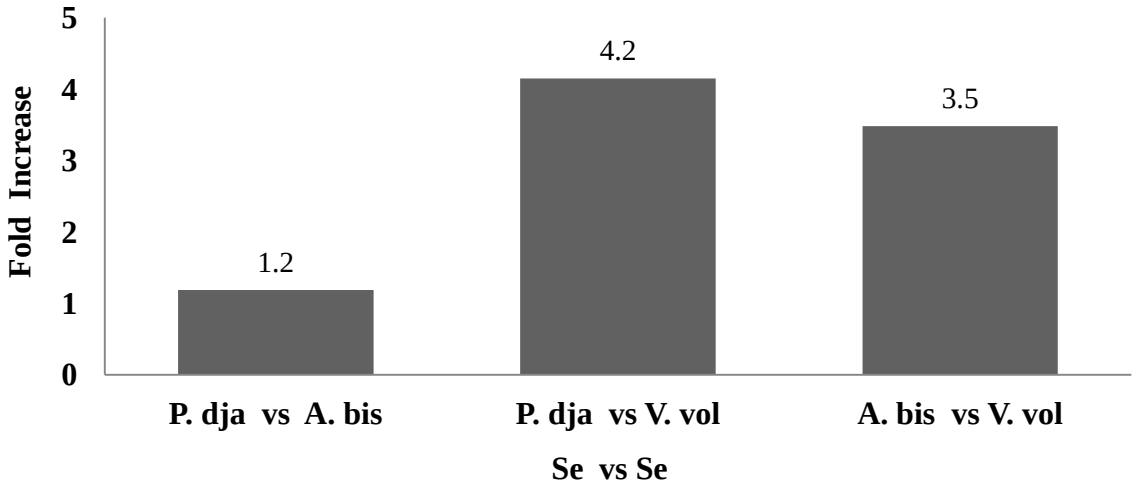


Figure 4.7. Comparitive study of selenium accumulation in terms of fold increase between Se-rich fruiting belonging to three different genera

To validate the Se content in mushrooms analyzed by florescence spectroscopy, total selenium content in the cultivation substrates (*i.e.*, wheat grain and straw) and in fruiting bodies of *Pleurotus florida* was measured using ICP-MS. Selenium content (Table 4.4) in Se-rich wheat substrates (27.9 ± 0.1 µg Se/g DW) was found be higher than the selenium concentration of control substrate (0.026 ± 0 µg/g DW). The fruiting bodies of *P. florida* harvested from Se-rich wheat straw (27.9 ± 0.1 µg Se/g DW) were noted to accumulate very high selenium content (141.0 ± 2.0 µg Se/g DW) as compared to control mushrooms (0.17 ± 0.01 µg Se/g DW) grown on control wheat straw (0.026 ± 0 µg Se/g DW). Se-rich fruiting bodies exhibited almost 800

times higher selenium content than the control mushroom fruiting bodies. The selenium content in growth substrates obtained using ICP-MS (27.9 ± 0.1 µg Se/g DW) was nearly comparable with those analyzed by florimetry (24.0 ± 0.2 µg Se/g DW). However total selenium content in Se-rich fruiting bodies of *P. florida* analyzed by ICP-MS (141 ± 2.0 µg Se/g DW) was found to be relatively higher than estimated by florimetry (110.5 ± 1.18 µg Se/g DW).

Table 4.4. Total selenium content in experimental (Se-enriched) substrates and resulting mushrooms with respect to controls using ICP-MS (n=3)

	Total selenium (µg Se/gDW)	
Sample	**Wheat straw**	**Mushroom**
Se	27.9 ± 0.1	141± 2.0
NSe	0.026	0.17 ± 0.01

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4.3 Selenium bioaccessiblity in mushrooms

This investigation was aimed at studying the bioaccessibility of selenium in Se-rich *P. florida* mushroom subjected to *in-vitro* gastrointestinal digestion (GI). Total selenium content in GI extracts was analyzed with the help of ICP-MS and is presented in Table 4.5. The soluble extract obtained after *in-vitro* simulated gastrointestinal digestion of the selenized mushroom contained about 106 ± 1.6 µg Se/g DW. It implied that the total soluble fraction of selenium simulating human gastrointestinal digestion was found to be 75% of the total selenium content obtained.

The GI extracts were subjected to size exclusion chromatography coupled to HPLC-ICP-MS to study the first fractionation of Se-species on the basis of molecular weight. The extracts investigated by size exclusion HPLC-ICP-MS revealed that proteins and other high molecular

weight selenium-containing molecules were hydrolyzed to peptides and low molecular weight selenocompounds i.e.≤ 5 kDa (Fig. 4.8) based on column calibration. A peak coeluting with a SeMet standard (retention time 36.5 min) was clearly visible in the size exclusion chromatogram, whereas the other major peak (retention time 34.6 min) likely corresponded to SeMet-selenoxide (SeOMet) and Se-peptides unspecifically produced by the gastrointestinal juice.

Table 4.5. Concentration of Se species in mushroom gastrointestinal hydrolysates determined by anion exchange HPLC-ICP-MS (µg Se/g DW) (n=3)

Sample	SeMet	Se(IV)	∑Other species [a]	Overall sum of species [b]	Total Se [c]
GI extract	69.2 ± 5.6	1.84 ± 0.23	23.6 ± 1.7	94.6 ± 5.9	105.8 ± 1.6

[a] Sum of Se species other than SeMet and Se(IV)
[b] Sum of SeMet, Se(IV) and ∑Other species
[c] As measured by ICP-DRC-MS.

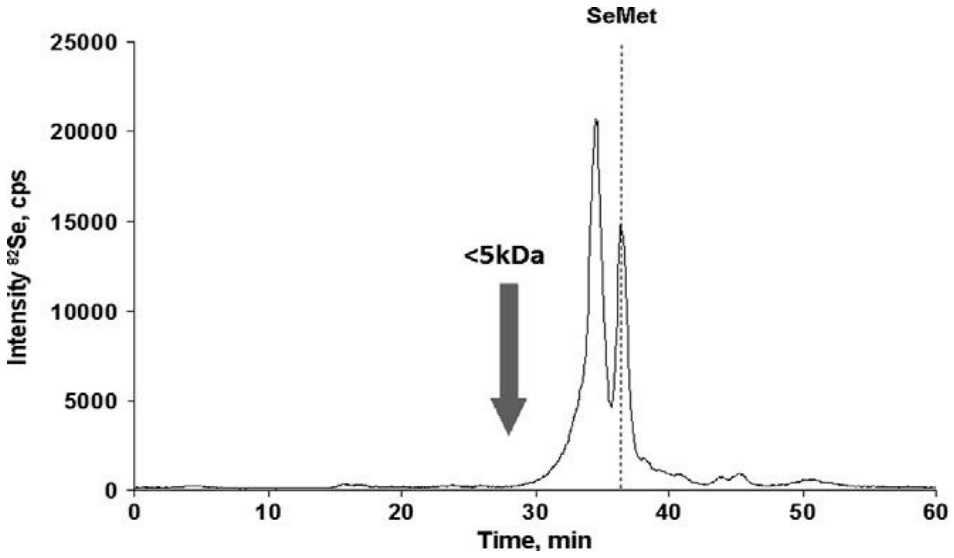


Figure.4.8. Selenium molecular fractions in a mushroom gastrointestinal hydrolysate: size exclusion HPLC-ICP-MS chromatogram

To study the speciation of bioaccessible forms of selenium liberated by GI extraction, anion exchange chromatography coupled to HPLC-ICP- MS was employed. The total (overall) sum of all selenium species detected in GI extracts was found to be 94.6 ± 5.9 µg Se/g DW. The major bioaccessible form in the GI extracts of Se-biofortified *P. florida* was found to be selenomethioneine (SeMet) exhibiting a concentraton of 69.2 ± 5.6 µg Se/g DW, which accounted for 73% of the sum of total species detected. A small percentage of SeMet (2%) was present as SeOMet which was accessed by addition of 0.5% v/v 2-mercaptoethanol (reducing agent that increased the SeMet peak) to the extracts (Fig. 4.9). Apart from these organic moieties, a little fraction (2 %) of inorganic Se was present in the form of Se (IV) exhibiting a concentration of 1.84 ± 0.23 µg Se/g DW. In addition to that, some unidentified low molecular weight selenocompounds with an overall concentration of 23.6 ± 1.7 µg Se/g DW were also detected which accounted for 25% of the sum of total species detected.

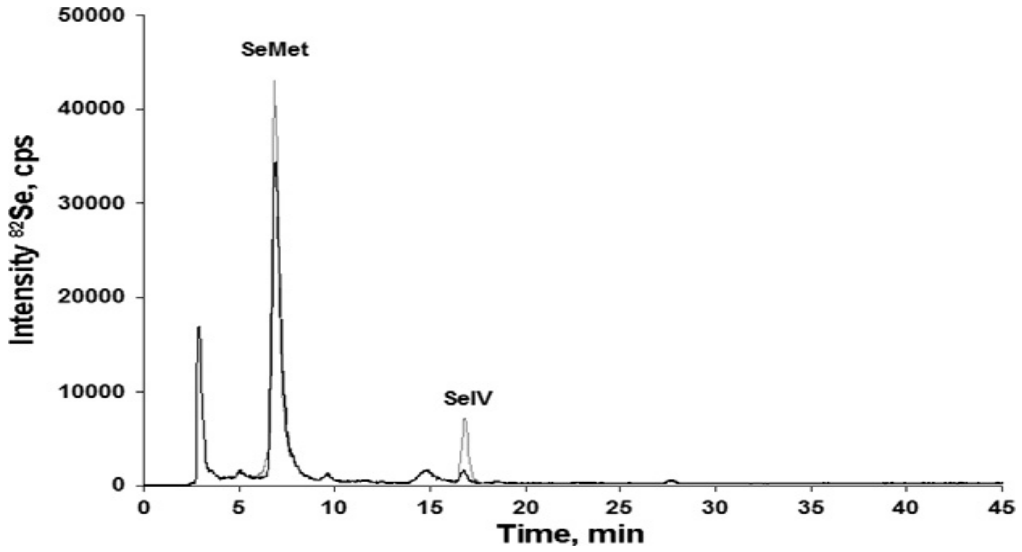


Figure 4.9. Selenium species in a mushroom gastrointestinal hydrolysate: anion exchange HPLC -ICP-MS chromatogram. Overlay of the chromatograms of the sample and the sample spiked with 250 and 50 µgSe/L of SeMet (RT ~6.5 min) and Se(IV) (RT ~17 min), respectively

The next section of work was planned to understand the modulations induced by selenium on various bioactive and anti-oxidant properties in extracts of fruiting bodies harvested from Se-rich substrate. The same analysis was followed for non enriched mushrooms (as control) cultivated on normal agricultural residues.

4.4 Elemental and protein content in mushrooms

4.4.1 Elemental analysis

The organic composition i.e. carbon (C), hydrogen (H) and nitrogen (N) content, expressed as percent dry weight of fruiting bodies was analyzed in selected oyster mushroom species cultivated on Se-rich and control substrates. In general the CHN content was nearly the same in fruiting bodies of all selenium enriched and control mushrooms (Table 4.6).

Table. 4.6. CHN content of Se-rich mushrooms as compared to control mushrooms

S.no	Mushroom	Carbon	Hydrogen	Nitrogen
1.	*Pleurotus djamor (Se)*	39.5	6.30	5.79
2.	*Pleurotus djamor (NSe)*	40.0	6.96	7.00
3.	*Pleurotus sajorcaju (Se)*	39.9	5.80	6.95
4.	*Pleurotus sajorcaju (NSe)*	38.7	5.80	7.18
5.	*Pleurotus citrinopileatus (Se)*	38.7	2.90	6.00
6.	*Pleurotus citrinopileatus (NSe)*	39.1	4.30	6.38
7.	*Pleurotus ostreatus (Se)*	38.8	3.50	5.77
8.	*Pleurotus ostreatus (NSe)*	38.9	4.60	6.55
9.	*Pleurotus fossulatus (Se)*	39.1	3.57	6.88
10.	*Pleurotus fossulatus(NSe)*	39.1	2.85	6.44
11.	*Pleurotus florida (Se)*	38.8	3.50	6.53
12.	*Pleurotus florida (NSe)*	38.5	4.97	6.44

Results inferred no significant difference between Se-fortified and control mushrooms, indicating that the Se fortification does not affect the nature of CHN profile of the mushrooms.

4.4.2 Effect of Se uptake on the absorption of other elements

To study the effect of selenium enrichment on the absorption of certain nutritionally important elements in mushrooms, the elemental (Fe, Zn, Mg, Ca and Cu) content of all seven species was studied. Among all metals detected, the order of accumulation by mushrooms was found to be highest for Mg in the range of 0.92 - 1.99 mg/g DW, followed by Ca (0.16 - 0.86 mg/g DW) ; Fe (0.1 - 0.52 mg/g DW); Zn (0.07 - 0.26 mg/g DW) and Cu (0.02 – 0.13 mg/g DW). All mushrooms belonging to both experimental and control were found to be good sources of minerals. *V. volvacea* was rich in Fe, Cu and Ca content, on the other hand *P. djamor* and *P. florida* were found to be rich in Mg and Zn. In general, the uptake behaviour for all elements of interest by Se-rich mushrooms varied within species but was found to be comparable with that of control mushrooms. Presence of selenium did not induce any appreciable change towards the profile of other elements that, in general, account for nutritional status of mushrooms.

4.4.3 Total protein content in mushrooms

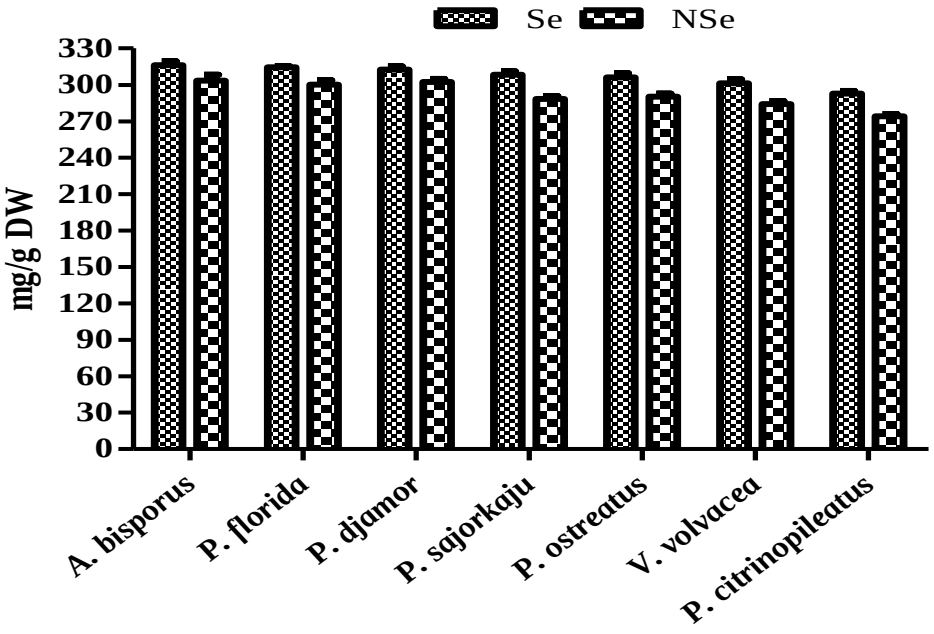
The average protein content of all mushrooms belonging to both Se and NSe (control) was studied and is represented in Table 4.7. In general, methanolic extracts belonging to both experimental (Se-rich) and control mushrooms showed appreciably higher protein content in fruiting bodies, however when compared amongst each other, Se-rich fruiting bodies of all seven species showed significantly higher ($P < 0.001$) protein yield over control. It was observed that all Se-rich species exhibited near one fold increase in protein content over the control species.

Among different species, the order of increase in terms of protein yield was found to be maximum for *A.bisporus* followed by *Pleurotus* sp. and *Volvariella.* Se-rich fruiting of *A.bisporus* showed significantly higher protein (316.0 ± 3.16 mg/g DW) content than its respective control (303.5 ± 4.92 mg/g DW). Similarly, among different *Pleurotus* species, Se-rich *P. florida* showed maximum protein content (314.4 ± 1.18 mg/g DW), followed by *P. djamor* (312.3 ± 2.87 mg/g DW), *P. sajorkaju* (308.4 ± 2.69 mg/g DW), *P. ostreatus* (306.3 ± 3.12 mg/g DW) and *P. citrinopileatus* (292.7 ± 2.2 mg/g DW) and these values (mg/g DW) were significantly higher over their respective controls (Table 4.7). Similar pattern was observed with Se-rich *V. volvacea* (301.1 ± 3.41 mg/g DW) showing significantly higher protein content over control (284.2 ± 2.29 mg/g DW). A multiple comparison analysis (Fig. 4.10) for protein yield was drawn between all Se-rich mushrooms. A significant relationship was obtained at $P< 0.05$ between different species of Se-rich mushrooms proving that these species possess varying protein content depending on their selenium concentration.

Table 4.7. Total protein content in experimental (Se-enriched) mushrooms and their respective controls (n=3)

Sample	Total protein (mg/g dw)						
	A. bisporus	***P. florida***	***P. sajorkaju***	***P. ostreatus***	***P. djamor***	***P. citrinopileatus***	***V. volvacea***
Se	316.0±3.16	314.4±1.18	308.4±2.69	306.3±3.12	312.3±2.87	292.7±2.2	301.1±3.41
NSe	303.5±4.92	300.0±3.65	288.2±2.54	290.2±2.38	302.3±2.48	274.1±1.78	284.2±2.29
	***	***	***	***	***	***	***

(***: P<0.001)



One way ANOVA between various Se rich species (*** at P < 0.05) Multiple comparison analysis		
P < 0.001	P < 0.01	P < 0.05
P.djamor vs P.citrinopileatus	***P.djamor vs V.volvacea***	***P.florida vs P.ostreatus***
P.florida vs V. volvacea		***A.bisporus vs P.ostreatus***
P.florida vs P.citrinopileatus		***V. volvacea vs P. citrinopileatus***
P.sajorkaju vs P. citrinopileatus		
P. citrinopileatus vs P.ostreatus		
A.bisporus vs V.volvacea		
A.bisporus vs P.citrinopileatus		

Figure 4.10. Total protein content in experimental (Se-enriched) mushrooms and their respective controls (n=3)

4.5 Antioxidant properties of mushrooms

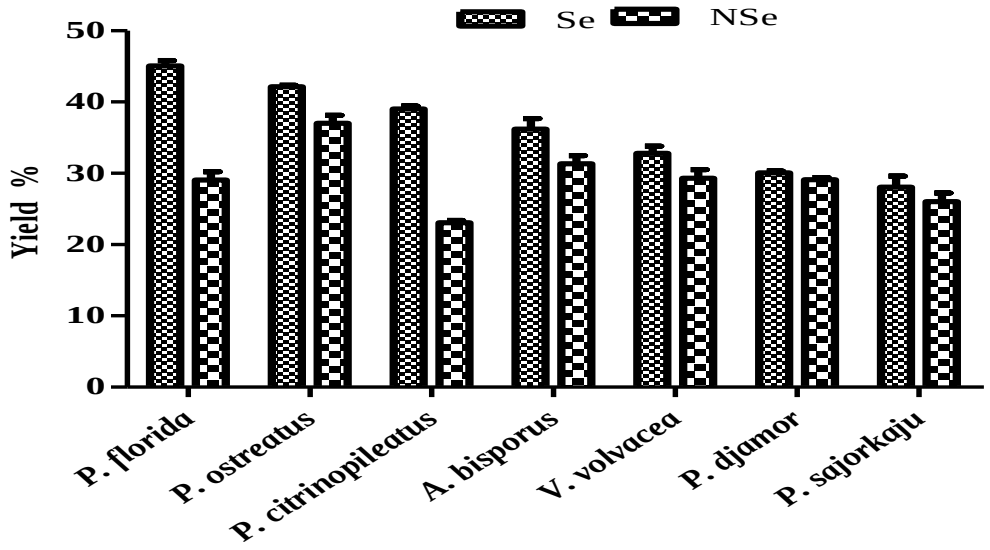
4.5.1 Yield of methanolic extracts

To evaluate the antioxidant status of mushrooms, extraction of fruiting bodies was carried out in 50% methanol. The percent extraction yield was recorded for each mushroom and is represented in Table 4.8. In general, the extraction yield of mushrooms belonging to both Se-rich and control was found to be effectively higher. Se-rich mushrooms showed significantly ($P< 0.05$ to $P< 0.001$) higher extraction yield over control mushrooms. Among different species, the order of decrease was for *P. florida* > *P. ostreatus* > *P. citrinopileatus* > *A. bisporus* > *V. volvacea* > *P. djamor* > *P. sajorkaju*. The extraction yields (%) among *Pleurotus* species, i.e. *P. florida*, *P.ostreatus*, *P. citrinopileatus*, *P.djamor* and *P. sajorkaju* were found to be 45.0 ± 0.76 ; 42.3 ± 0.2; 39.0 ± 0.43; 30.0 ± 0.21 and 28.0 ± 1.57 which were significantly higher over their respective controls (29.0 ± 1.18; 37.0 ± 1.1; 23.0 ± 0.32; 29.1 ± 0.27 and 26.0 ± 2.54%). Similarly, Se-rich *A.bisporus* (36.1 ± 1.5 %) and *V. volvacea* (32.7 ± 1.0 %) showed significantly higher yield as compared to their respective controls (31.3 ± 1.1 and 29.3 ± 1.2 %). It was observed that all Se-rich species exhibited 1.0 - 1.6 fold increase in extraction yield over the control species.

Table 4.8. Total yield of mushroom extracts in selenium enriched mushrooms and their respective controls (n=3)

Sample	Yield of extracts (%)						
	A. bisporus	***P. florida***	***P. sajorkaju***	***P. ostreatus***	***P. djamor***	***P. citrinopileatus***	***V. volvacea***
Se	36.1 ± 1.5	45.0 ± 0.76	28.0 ± 1.57	42.3 ± 0.2	30.0 ± 0.21	39.0 ± 0.43	32.7 ± 1.0
NSe	31.3 ± 1.1	29.0 ± 1.18	26.0 ± 2.54	37.0 ± 1.1	29.1 ± 0.27	23.0 ± 0.32	29.3 ± 1.2
	*	***	*	**	**	***	**

A multiple comparison analysis (Fig. 4.11) for extraction yield was drawn between all Se-rich mushrooms. A statistically significant relationship was obtained at P< 0.05 between different species of Se-rich mushrooms proving that different Se-rich species possess different extraction yield as function of selenium concentration in respective matrices.



One way ANOVA between various Se rich species (*** at P < 0.05) Multiple comparison analysis		
P < 0.001	P < 0.01	P < 0.05
P. florida **vs** ***V.volvacea*** ***P.ostreatus*** **vs** ***V.volvacea*** ***P. citrinopileatus*** **vs** ***V.volvacea*** ***A. bisporus*** **vs** ***V.volvacea*** ***V.volvacea*** **vs** ***P. djamor*** ***V.volvacea*** **vs** ***P.sajorkaju***	***P. florida*** **vs** ***P. djamor*** ***P. florida*** **vs** ***P.sajorkaju*** ***P.ostreatus*** **vs** ***P.sajorkaju***	***P.citrinopileatus*** **vs** ***P.sajorkaju*** ***P.ostreatus*** **vs** ***P. djamor***

Figure 4.11. Total yield of mushroom extracts in selenium enriched mushrooms and their respective controls (n=3)

4.5.2 Total phenol content

Selenium is well known for antioxidant reaction in humans and animals. So, it is important to consider the effect of the total phenol content on the antioxidant activity of selenium rich mushroom extracts. The total phenolic content, expressed as mg of GAE/g of dry mushroom, is shown in Table 4.9. The amount of phenolic compounds in the methanolic extracts from the Se-enriched mushrooms was found to be significantly higher ($P < 0.01$ to $P < 0.001$) than their respective control. Se-rich fruiting bodies (17.73 ± 0.5 mg GAE/g DW) of *V. volvacea* showed significantly higher phenol content than control (12.7 ± 0.5 mg GAE/g DW). Among *Pleurotus* genus, Se-rich species of *P. djamor* (13.25 ± 0.57 mg GAE/g DW), *P. citrinopileatus* (10.5 ± 0.5 mg GAE/g DW), *P. sajorkaju* (8.39 ± 0.65 mg GAE/g DW), *P. florida* (7.9 ± 0.3 mg GAE/g DW) and *P. ostreatus* (7.34 ± 0.30 mg GAE/g DW) also exhibited significantly higher phenol content than their respective control (Table 4.9). Similar pattern was observed for Se-rich species of *A. bisporus* over control.

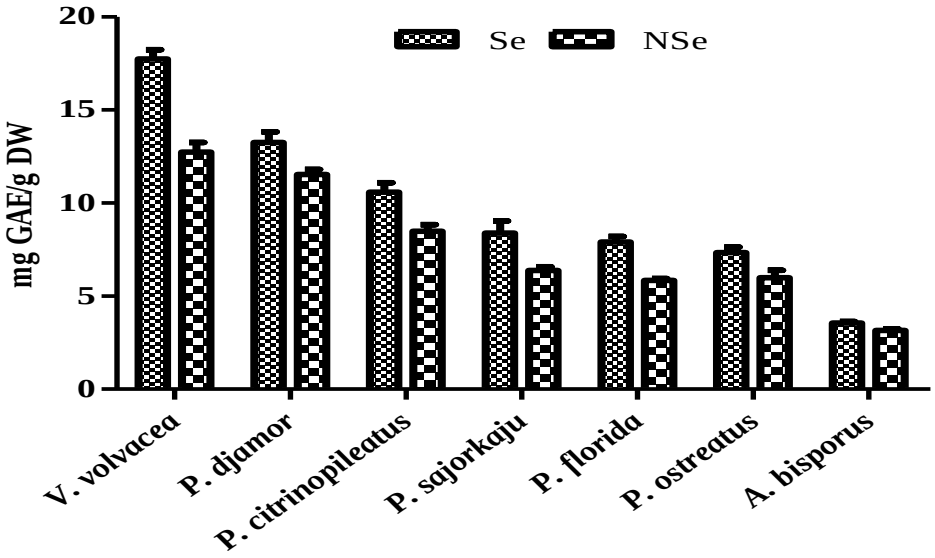
Table 4.9. Total phenol profiles in fruiting bodies Se-enriched and corresponding control mushrooms. (n = 3)

Sample	Total phenols (mg GAE/g DW)						
	A. bisporus	***P. florida***	***P. sajorkaju***	***P. ostreatus***	***P. djamor***	***P. citrinopileatus***	***V. volvacea***
Se	3.55 ± 0.08	7.9 ± 0.30	8.39 ± 0.65	7.34 ± 0.30	13.25 ± 0.57	10.59 ± 0.50	17.73 ± 0.5
NSe	3.15 ± 0.09	5.9 ± 0.39	6.37 ± 0.20	5.90 ± 0.39	11.54 ± 0.26	8.49 ± 0.35	12.73 ± 0.5
	**	**	**	**	**	**	***

(**: P<0.01; ***: P<0.001)

It was observed that all Se-rich species exhibited almost one fold increase in total phenol content over the control species. The phenol content when compared in terms of Se vs NSe, was increasing as a function of high selenium concentration in enriched mushrooms against low

selenium concentration in NSe mushrooms. Comparison drawn among various species indicated significant difference across all Se-rich mushroom species studied. A significant relationship was observed at $P < 0.05$ in terms of phenol content between Se-rich species when subjected to multiple comparison (Tukey test) analysis using one way ANOVA (Fig. 4.12).



One way ANOVA between various Se rich species	
(*** at $P < 0.05$)	
Multiple comparison analysis	
$P < 0.001$	
V.volvacea* vs *P. djamor	***P. djamor* vs *P.ostreatus***
V.volvacea* vs *P. citrinopileatus	***P. djamor* vs *A.bisporus***
V.volvacea* vs *P. sajorkaju	***P. citrinopileatus* vs *P. sajorkaju***
V.volvacea* vs *P. florida	***P. citrinopileatus* vs *P. florida***
V.volvacea* vs *P.ostreatus	***P. citrinopileatus* vs *P.ostreatus***
V.volvacea* vs *A.bisporus	***P. citrinopileatus* vs *A.bisporus***
P. djamor* vs *P. citrinopileatus	***P. sajorkaju* vs *A.bisporus***
P. djamor* vs *P. sajorkaju	***P. florida* vs *A.bisporus***
P. djamor* vs *P. florida	***P.ostreatus* vs *A.bisporus***

Figure 4.12. Total phenol profiles in fruiting bodies Se-enriched and corresponding control mushrooms. (n = 3)

However, statistical studies between Se-rich *P. sajorkaju* vs *P. ostreatus*, *P. sajorkaju* vs *P. florida* and *P. florida* vs *P. ostreatus* were found to be non-significant for comparative phenol content. It implies that phenol content varies with selenium concentration in fruiting bodies across all Se-rich species.

4.5.3 Total antioxidant activity

Total antioxidant content of the methanol extracts of mushrooms was measured spectrophotometrically through phosphomolybdenum assay and was expressed as mg BHT/g DW (Table 4.10). The antioxidant activity of Se-rich mushroom extracts was found to be significantly higher ($P < 0.05$ to $P < 0.001$) than their respective control. Among five Se-rich *Pleurotus* species, *P. djamor* (5.13 ± 0.15) showed significantly higher antioxidant content followed by *P. sajorkaju* (4.58 ± 0.16 mg BHT/g DW); *P. florida* (4.15 ± 0.23 mg BHT/g DW); *P. ostreatus* (3.50 ± 0.19 mg BHT/g DW) and *P. citrinopileatus* (2.37 ± 0.10 mg BHT/g DW) and were found to be higher than their respective controls (Table 4.10). Similarly, Se-rich *A. bisporus* (4.37 ± 0.3 mg BHT/g DW) and *V. volvacea* (0.93 ± 0.01 mg BHT/g DW) exhibited significantly higher antioxidant content in respect to their controls (2.50 ± 0.3; 0.82 ± 0.07 mg BHT/g DW).

Table 4.10. Total antioxidant content in selenium enriched mushrooms and their respective controls (n=3)

Sample	Total antioxidant content (mg/g DW)						
	A. bisporus	***P. florida***	***P. sajorkaju***	***P. ostreatus***	***P. djamor***	***P. citrinopileatus***	***V. volvacea***
Se	4.37 ± 0.3	4.15 ± 0.23	4.58 ± 0.16	3.50 ± 0.19	5.13 ± 0.15	2.37 ± 0.10	0.93 ± 0.01
NSe	2.50 ± 0.3	3.0 ± 0.33	1.45 ± 0.23	2.80 ± 0.18	3.90 ± 0.34	1.81 ± 0.08	0.82 ± 0.07
	**	**	***	*	**	**	***

It was observed that all Se-rich species exhibited almost 1-3 fold increase in total antioxidant content over the control species (Fig. 4.13). The total antioxidant content among Se-rich species in terms of fold increase was also determined, wherein *P. djamor* showed the highest antioxidant content followed by *A. bisporus* and *V. volvacea*. *P. djamor* showed 1.8 and 5.5 times higher antioxidant content over *A. bisporus* and *V. volvacea* respectively, whereas *A. bisporus* showed almost 4.6 fold increase in antioxidant levels over *V. volvacea*. Statistical variation ($P < 0.05$ to $P < 0.001$) in terms total antioxidant content was observed, among various Se-rich species (Fig. 4.14). However, analysis between Se-rich *P. djamor* vs *P. sajokaju*, *P. sajorkaju* vs *A. bisporus*, *P. sajorkaju* vs *P. florida* and *A. bisporus* vs *P. florida* were found to be non-significant for comparative antioxidant content.

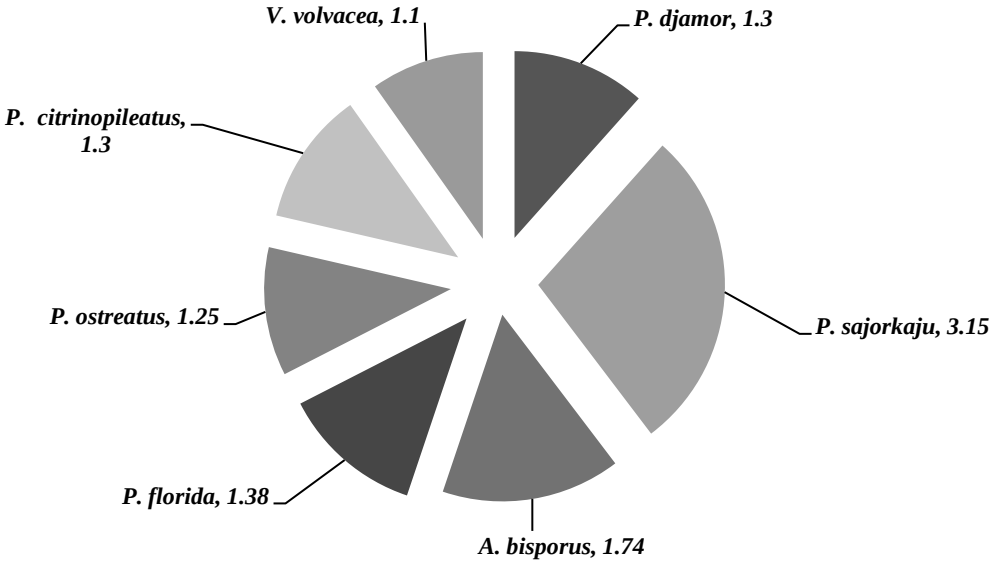
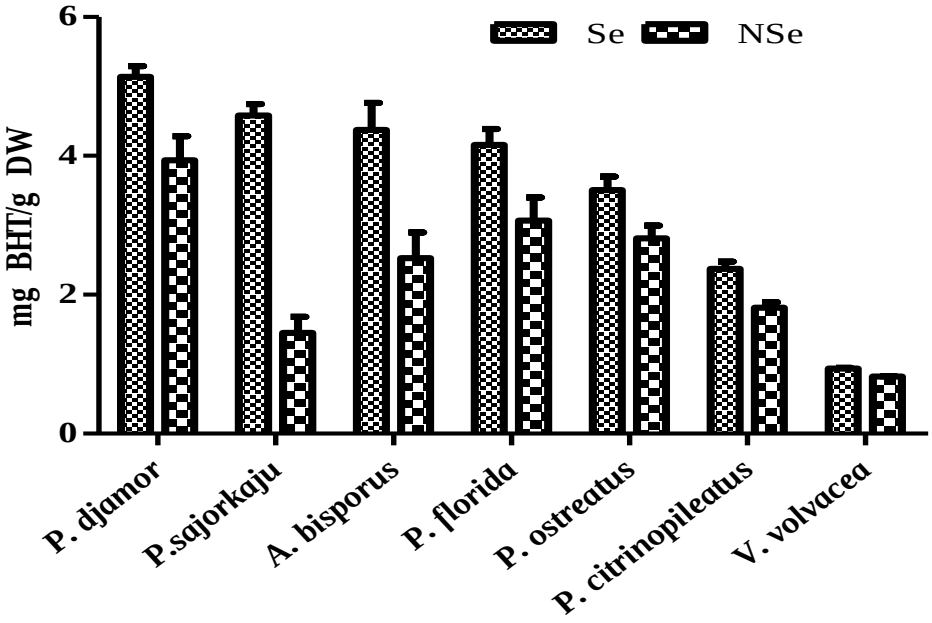


Figure 4.13. Total antioxidant content in Se-rich fruiting bodies in terms of fold increase over control (NSe)



One way ANOVA between various Se rich species (*** at P < 0.05) Multiple comparison analysis		
P < 0.001	P < 0.01	P < 0.05
P.djamor* vs *P.florida ***P.djamor* vs *P.ostreatus*** ***P.djamor* vs *P.citrinopileatus*** ***P.djamor* vs *V.volvacea*** ***P.sajorkaju* vs *P.ostreatus*** ***P.sajorkaju* vs *P.citrinopileatus*** ***P.sajorkaju* vs *V.volvacea*** ***A.bisporus* vs *P.citrinopileatus*** ***A.bisporus* vs *V.volvacea*** ***P.florida* vs *P.citrinopileatus*** ***P.florida* vs *V.volvacea*** ***P.ostreatus* vs *P.citrinopileatus*** ***P.ostreatus* vs *V.volvacea*** ***P.citrinopileatus* vs *V.volvacea***	***P.djamor* vs *A.bisporus*** ***A.bisporus* vs *P.ostreatus***	***P.florida* vs *P.ostreatus***

Figure 4.14. Total antioxidant content in selenium enriched mushrooms and their respective controls (n=3)

4.5.4 Inhibition of lipid peroxidation

Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) upon decomposition. MDA is the secondary byproduct, which is released during lipid peroxidation (LPO). This study was conducted to analyze the influence of Se-rich fraction of mushrooms on inhibition of LPO in terms of MDA production. A decrease in production of MDA symbolized the inhibition of LPO. Se-rich mushrooms in general showed significantly lower values of MDA as compared to control mushrooms (Table 4.11). The selenium rich extracts of all mushrooms showed significant decrease ($P < 0.001$) in MDA levels as compared to their respective control.

The decreasing pattern of LPO inhibition exhibited by Se-rich mushrooms was found to be in the order *A. bisporus* < *P. sajorkaju* (lowest among *Pleurotus* species) < *V. volvacea*. Among Se-rich *Pleurotus* species, *P. sajorkaju* exhibited relatively lowest (17.6 ± 4.4 nM MDA/g DW) MDA levels followed by *P. ostreatus* (36.2 ± 2.32 nM MDA/g DW); *P. citrinopileatus* (115.6 ± 2.41 nM MDA/g DW); *P. djamor* (121.5 ± 2.36 nM MDA/g DW) and *P. florida* (159.8 ± 2.37 nM MDA/g DW) (Table 4.11). Similarly, Se-rich *A. bisporus* (10.72 ±3.6 nM MDA/g DW) and *V. volvacea* (475.7 ± 4.7 nM MDA/g DW) showed significantly lower MDA levels than their respective controls (Table 4.11).

It was observed that all Se-rich species exhibited about 1 - 26 fold decrease in MDA levels over the control species (Fig.4.15). When Se-rich species were compared for relative inhibition of LPO among each other, a statistically significant variation (multiple comparisons) was observed at $P < 0.05$ (Fig. 4.16). However, statistical studies between Se-rich *P. sajokaju* vs *A. bisporus*, *P .citrinopileatus* vs *P. djamor* were found to be in-significant for comparative MDA levels.

Table 4.11. Lipid peroxidation activity (TBARS) of extracts from Se enriched and control fruiting bodies (n=3)

Sample	Lipid peroxidation (nM MDA/g DW)						
	A. bisporus	***P. florida***	***P. sajorkaju***	***P. ostreatus***	***P. djamor***	***P. citrinopileatus***	***V. volvacea***
Se	10.72 ± 3.6	159.8 ± 2.37	17.66 ± 4.41	36.25 ± 2.32	121.5 ± 2.36	115.6 ± 2.41	475.7 ± 4.7
NSe	32.0 ± 1.60	185.1 ± 2.27	439.2 ± 4.19	64.60 ± 2.32	241.5 ± 5.33	216.3 ± 1.92	640.6 ± 5.2
	***	***	***	***	***	***	***

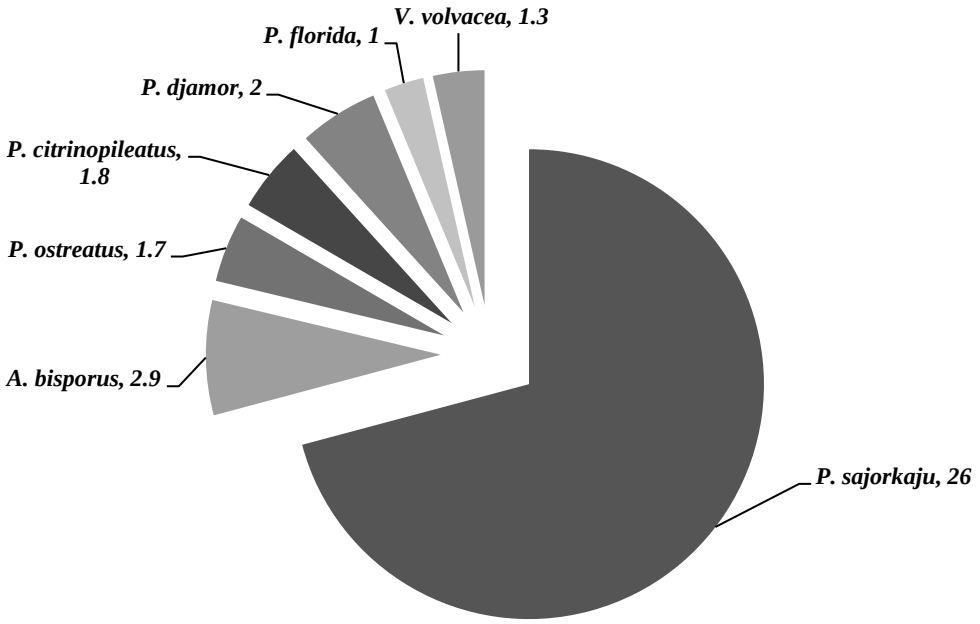
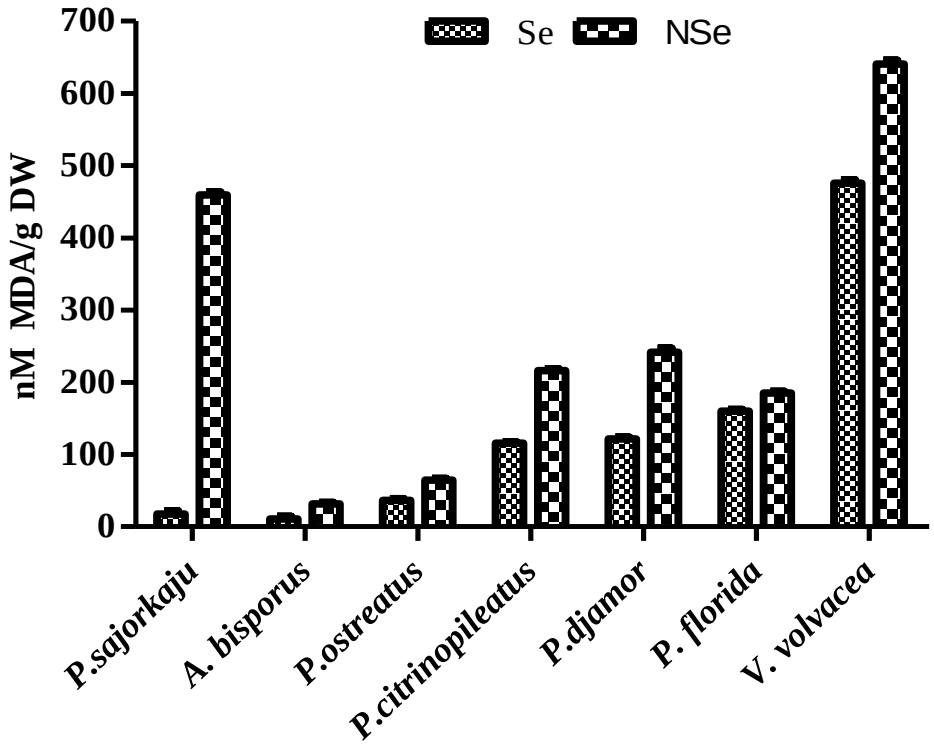


Figure 4.15. Inhibition of Lipid peroxidation in Se-rich fruiting bodies in terms of fold increase over control (NSe)



One way ANOVA between various Se rich species	
(*** at P < 0.05)	
Multiple comparison analysis	
P < 0.001	
P.sajorkaju vs P.ostreatus	***P.ostreatus vs P. citrinopileatus***
P.sajorkaju vs P. citrinopileatus	***P.ostreatus vs P. djamor***
P.sajorkaju vs P. djamor	***P.ostreatus vs P. florida***
P.sajorkaju vs P. florida	***P.ostreatus vs V.volvacea***
P.sajorkaju vs V.volvacea	***P. citrinopileatus vs P. florida***
A.biosporus vs P.ostreatus	***P. citrinopileatus vs V.volvacea***
A.biosporus vs P. citrinopileatus	***P. djamor vs P. florida***
A.biosporus vs P. djamor	***P. djamor vs V.volvacea***
A.biosporus vs P. florida	***P. florida vs V.volvacea***
A.biosporus vs V.volvacea	***P. florida vs V.volvacea***

Figure 4.16. Inhibition of lipid peroxidation (nM MDA/g DW) by extracts of Se-enriched and control mushrooms (n=3)

4.5.5 Free radical scavenging activity

To understand the modulations induced by selenium on anti-oxidant properties, the extracts of Se-rich fruiting bodies were subjected to free radical (DPPH) scavenging assay (FRS). DPPH scavenging activities of methanolic extracts of mushrooms were measured in different concentrations ranging from 0.05 to 15 mg/ml and results were expressed as percent DPPH scavenging (Table 4.12). The scavenging effect of methanolic extract of Se-enriched and control mushrooms increased with increasing concentration of extract; however Se-rich mushrooms showed significantly higher radical scavenging than their respective controls ($P < 0.05$ to $P < 0.001$). The highest percent DPPH scavenging ability was detected in methanolic extracts of Se-rich *A. bisporus* followed by *Pleurotus sp* and *V. volvacea*. At 1.0 mg/ml of Se-enriched extract of *A. bisporus*, the scavenging effect was found to be 91.4 ± 1.5% which was significantly higher when compared to control (84.9 ± 0.5%). Similarly among Se-rich bodies of *Pleurotus* species, at an concentration of 1.0 mg/ml, the radical scavenging was in the range of 13.5 ± 0.5 to 17.23 ± 0.7 % which was relatively higher than their respective controls (11.7 ± 0.8 to 14.3 ± 0.7 %). Se-rich *V. volvacea* at concentration of 1mg/ml also showed higher scavenging (15.4 ± 0.17 %) than control **(**10.3 ± 0.21%). It was observed that all Se-rich species exhibited almost 1-2.4 fold increase in radical scavenging over the control species (Fig. 4.17).

Concentration dependent scavenging ability of all mushrooms exhibiting low to moderate activities were also expressed in terms of EC_{50} (effective concentration to inhibit 50 % of FRS activity) and is represented in Table 4.13. In general the EC_{50} among all mushrooms (Se-rich and control) was found to be in range of 0.056 – 14.4 mg/ml, however the Se-rich mushroom extracts showed relatively lower EC_{50} than control (Table 4.13). Among Se-rich mushrooms, *A. bisporus* (0.056 mg/ml) showed lowest EC_{50} values than *Pleurotus* species (9.3 to

Table 4.12. DPPH scavenging potential of extracts from Se enriched and their respective control fruiting bodies (n=3)

Scavenging effect (%) of mushroom species on DPPH Methanolic extract (mg/ml)								
Sample	**0.05**	**0.1**	**0.2**	**0. 3**	**0.4**	**0.5**	**1.0**	
				A. bisporus				
Se	47.9 ± 0.09	64.1 ± 1.3	68.2 ± 0.7	72.8 ± 2.35	76.0 ± 1.61	78.0 ± 1.77	91.4 ± 1.49	
Non-Se	40.4 ± 1.0	59.8 ± 2.2	62.8 ± 1.2	66.9 ± 1.25	70.5± 1.42	70.5 ± 1.0	84.9 ± 0.59	
	*	*	**	*	*	*	**	
	0.5	**1**	**2**	**3**	**4**	**5**	**10**	**15**
				P. sajorkaju				
Se	14.0 ± 0.5	17.23 ± 0.7	22.5 ± 0.5	26.8 ± 0.52	37.7 ± 0.6	40.6 ± 1.0	51.8 ± 0.3	66.7 ± 1.0
Non-Se	11.1 ± 0.9	14.36 ± 0.7	19.7 ± 0.6	21.8 ± 0.32	28.9 ± 1.0	33.6 ± 0.7	45.1 ± 1.8	57.7±0.8
	**	**	**	***	***	***	**	***
				P. florida				
Se	12.6 ± 0.4	14.1 ± 0.4	23.5 ± 0.30	25.4 ± 0.60	33.2 ± 0.7	35.9 ± 0.6	50.2 ± 0.8	64.3 ± 0.7
Non-Se	10.0 ± 1.1	12.0 ± 0.3	21.4 ± 0.09	23.1 ± 0.41	30.5 ± 0.2	32.6 ± 0.3	44.1 ± 1.4	56.2 ± 0.8
	*	**	***	**	**	**	**	***
				P. djamor				
Se	11.0 ± 0.6	13.5 ± 0.5	18.0 ± 0.5	25.4 ± 0.45	29.7 ± 0.5	32.1 ± 0.9	46.3 ± 1.2	61.0 ± 0.5
Non-Se	9.4 ± 0.36	11.9 ± 0.3	16.5 ± 0.5	23.5 ± 0.50	26.8 ± 0.6	30.3 ± 0.4	41.9 ± 0.8	55.7 ± 0.6
	*	*	*	**	**	*	**	***
				P. citrinopileatus				
Se	13.7 ± 0.2	16.5 ± 0.3	18.6 ± 0.5	23.1 ± 0.54	24.9 ± 0.60	29.6 ± 0.3	46.4 ± 0.4	60.0 ± 0.5
Non-Se	10.0 ± 0.5	12.8 ± 0.6	15.5 ± 0.8	20.5 ± 0.55	23.5 ± 0.60	29.0 ± 0.9	44.8 ± 0.6	56.0 ± 0.4
	***	***	**	**	*	ns	*	***
				P. ostreatus				
Se	11.9 ± 0.5	14.1 ± 0.5	18.5 ± 0.7	24.4 ± 0.75	28.8 ± 0.93	33.8 ± 0.5	43.1 ± 1.2	55.0 ± 0.6
Non-Se	9.20 ± 0.4	11.7 ± 0.8	15.6 ± 0.7	19.4 ± 0.69	23.3 ± 0.98	28.7 ± 1.0	37.3 ± 1.1	50.2 ± 0.5
	**	*	**	**	**	**	**	***
				V. volvacea				
Se	10.9 ± 0.1	15.4 ± 0.17	29.6 ± 0.43	37.9 ± 1.22	55.2 ± 0.76	62.0 ± 0.48	71.1± 0.8	80.0 ± 0.4
Non-Se	4.5 ± 0.3	10.3 ± 0.21	24.1 ± 0.89	32.1 ± 1.65	47.4 ± 0.61	57.1 ± 0.58	65.1 ± 0.5	76.3 ± 0.6
	***	***	***	**	***	**	**	**
BHA[@]	96.7 ± 0.5	-	-	-	-	-	-	-
Two-way ANOVA								
Comparison between extract concentrations: significant at p<0.001								

(*: P<0.05; **: P<0.01; ***: P<0.001; ns-non-significant)

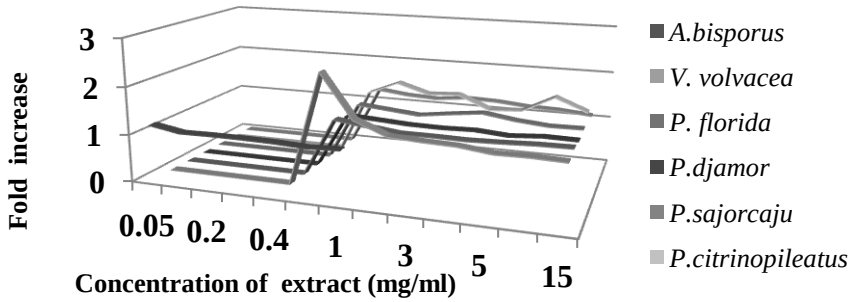


Figure 4.17. Free radical Scavenging in Se-rich fruiting bodies in terms of fold increase over control (NSe)

12.4 mg/ml) and *V. volvacea* (6.0 mg/ml). The statistical tests of significance on free radical scavenging showed no significant difference at P > 0.05 between Se-rich species (Fig. 4.18), However, when compared between various extract concentrations (P <0.05), it was observed that free radical scavenging for a particular species increased with increasing concentration.

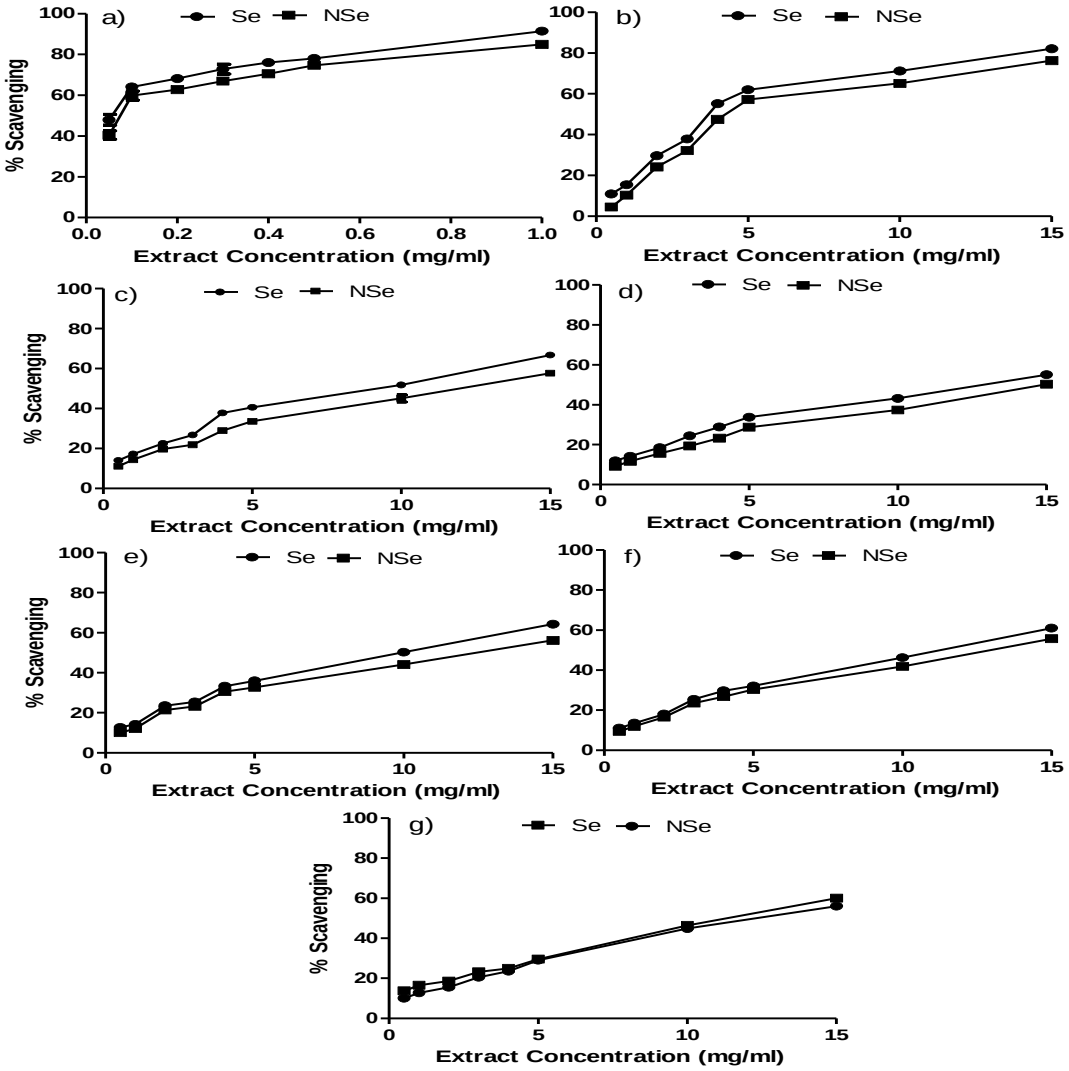


Figure 4.18. DPPH scavenging (%) potential of methanolic extracts of Se-rich mushrooms; a) *A. bisporus,* b) *V. volvacea,* c) *P. sajorkaju,* d) *P. ostreatus,* e) *P. florida,* f) *P. djamor,* g) *P. citrinopileatus*; as compared to their respective controls (n=3)

Table 4.13. EC_{50} values obtained in the antioxidant activity (in terms of DPPH scavenging) of Se-rich mushrooms as compared to NSe mushrooms

DPPH scavenging (EC50 values; mg/ml)		
Sample	**Se**	**NSe**
A. bisporus	0.056	0.076
P. florida	9.90	11.90
P. sajorkaju	9.35	11.80
P. ostreatus	12.4	14.40
P. djamor	11.6	13.10
P. citrinopileatus	11.4	12.20
V. volvacea	6.0	7.30

4.5.6 Metal chelation activity

In the present study, the chelating ability of the mushroom extracts toward ferrous ions (Fe^{2+}) was investigated. The metal chelating ability of mushroom extracts was expressed in terms of percent metal chelation and is presented in Table 4.14. The percent chelation effect in Se-rich and control mushrooms increased with increasing concentration of extracts. The highest metal chelation activity at 10 mg/ml was exhibited by *P. florida* (97.1 ± 0.40%) followed by *A. bisporus* (93.7 ± 0.7%) and *V. volvacea* (56.0 ± 1.75 %). In general, Se-rich mushroom extracts exhibited significantly higher ($P < 0.05$ to $P < 0.001$) metal chelation than control mushroom extracts.

Among Se-rich fruiting bodies of *Pleurotus* species, at a concentration of 10 mg/ml, the metal chelation was in the range of (85.0 ± 0.34 - 97.1 ± 0.4 %) which was relatively higher than their respective control range (75.4 ± 0.73 – 86.0 ± 0.7 %). Similarly, Se-enriched extract of *A. bisporus* (93.7 ± 0.7%) and *V. volvacea* (56.0 ± 1.75 %) exhibited significantly higher metal

chelation then their respective control (78.2 ± 1.7 %and 43.0 ± 1.41 %). The chelating activities of mushroom extracts were determined by the ferrozine assay and the effectiveness of extracts at

Table 4.14. Metal chelating activity of extracts from Se enriched and their respective control fruiting bodies (n=3)

		Metal chelation (%) of mushroom species Methanolic extract (mg/ml)					
Sample	**0.4**	**1**	**2**	**3**	**4**	**5**	**10**
EDTA	**85.4 ± 0.7**						
				A. bisporus			
Se	-	10.9 ± 0.91	26.5 ± 0.98	39.0 ± 0.9	46.4 ± 2.83	53.4 ± 0.92	93.7 ± 0.7
Non-Se	-	1.0 ± 0.73 ***	4.40 ± 1.22 ***	16.6 ± 1.7 ***	28.7 ± 1.37 ***	34.0 ± 0.87 ***	78.2 ± 1.7 ***
				P. sajorkaju			
Se	-	19.0 ± 0 .96	45.0 ± 2.76	67.0 ± 1.81	73.2 ± 1.99	90.7 ± 1.37	95.4 ± 0.32
Non-Se	-	11.3 ± 1.31 ***	31.5 ± 0.54 **	45.1 ± 2.75 ***	59.8 ± 2.70 **	75.0 ± 1.96 ***	83.1 ± 2.85 **
				P. florida			
Se	-	10.4 ± 1.38	26.7 ± 1.36	49.7 ± 0.86	58.5 ± 0.42	75.0 ± 0.34	97.1 ± 0.40
Non-Se	-	5.90 ± 1.13 *	16.8 ± 1.80 **	35.1 ± 0.49 ***	47.9 ± 0.66 ***	65.9 ± 0.83 ***	86.0 ± 0.75 ***
				P. djamor			
Se	-	21.0 ± 0.87	40.8 ± 0.79	59.5 ± 0.79	81.7 ± 2.57	87.7 ± 0.88	96.6 ± 0.45
Non-Se	-	12.4 ± 1.70 **	31.2 ± 0.95 ***	44.3 ± 1.98 ***	59.2 ± 1.95 ***	63.9 ± 0.52 ***	85.5 ± 2.01 ***
				P. citrinopileatus			
Se	-	3.06 ± 0.34	12.7 ± 0.31	32.9 ± 0.53	53.0 ± 0.57	66.9 ± 0.82	85.0 ± 0.34
Non-Se	-	1.21 ± 0.20 **	8.21 ± 1.55 **	23.4 ± 1.03 ***	43.1 ± 0.31 ***	56.7 ± 0.34 ***	75.4 ± 0.73 ***
				P. ostreatus			
Se	-	3.43 ± 0.46	18.0 ± 1.27	31.2 ± 1.95	50.7 ± 0.14	62.3 ± 1.33	88.4 ± 1.08
Non-Se	-	1.1 ± 0.76 *	9.4 ± 0.93 ***	19.3 ± 1.84 ***	33.0 ± 1.11 ***	45.5 ± 1.30 ***	82.0 ± 0.50 ** *
				V. volvacea			
Se	-	15.0 ± 1.65	21.8 ± 1.41	32.0 ± 1.65	35.0 ± 1.75	47.0 ± 0.53	56.0 ± 1.75
Non-Se	-	10.0 ± 1.58 *	13.8 ± 1.41 **	24.2 ± 0.92 **	30.6 ± 0.88 *	36.0 ± 0.88 ***	43.0 ± 1.41 **

(*: P<0.05; **: P<0.01; ***: P<0.001; ns-non-significant)

concentrations ranging from 1-10 mg/ml was also expressed as EC_{50} and is presented in Table 4.15. The Fe^{2+}-ferrozine complex formation was significantly prevented by methanolic extracts of mushroom species. In general, the EC_{50} among all mushrooms (Se-rich and control) was found to be in range of 2.12 – 7.72 mg/ml, however the Se-rich mushroom extracts showed relatively lower EC_{50} than control. Among Se-rich *Pleurotus* species, *P. sajorkaju* (2.12 mg/ml)

showed lowest EC_{50} values than *A. bisporus* (4.7 mg/ml) and *V. volvacea* (7.72 mg/ml). It was observed that all Se-rich species exhibited almost 1 - 10 fold increase in metal chelation over the control species at varying concentrations (Fig. 4.19)

Table 4.15. EC_{50} values obtained in the antioxidant activity (in terms of metal chelation) of Se-rich mushrooms as compared to NSe mushrooms

Metal Chelation (EC_{50} values; mg/ml)		
Sample	**Se**	**NSe**
A. bisporus	4.70	6.80
P. florida	3.91	4.97
P. sajorkaju	2.12	4.0
P. ostreatus	4.95	6.22
P. djamor	2.34	4.28
P. citrinopileatus	5.01	6.0
V. volvacea	7.72	> 10

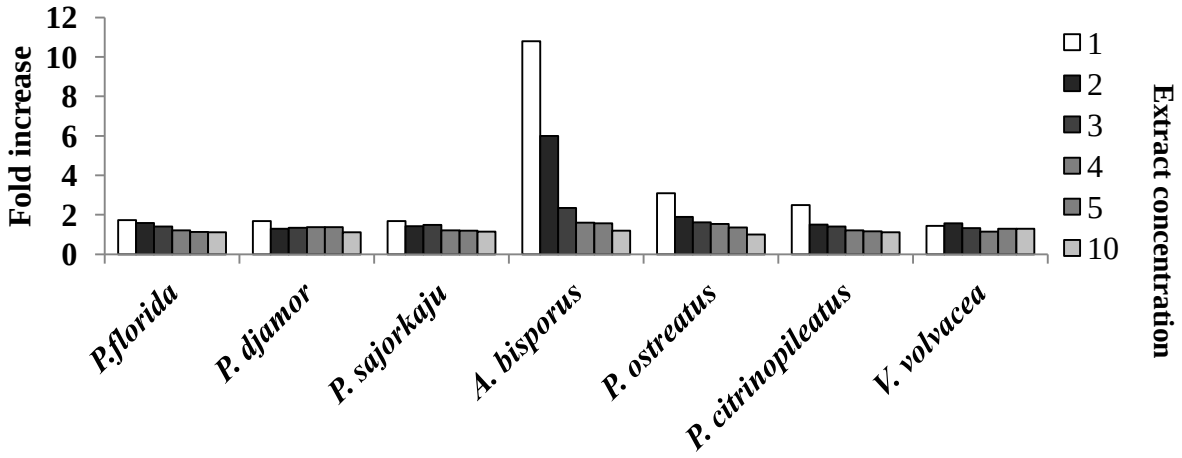
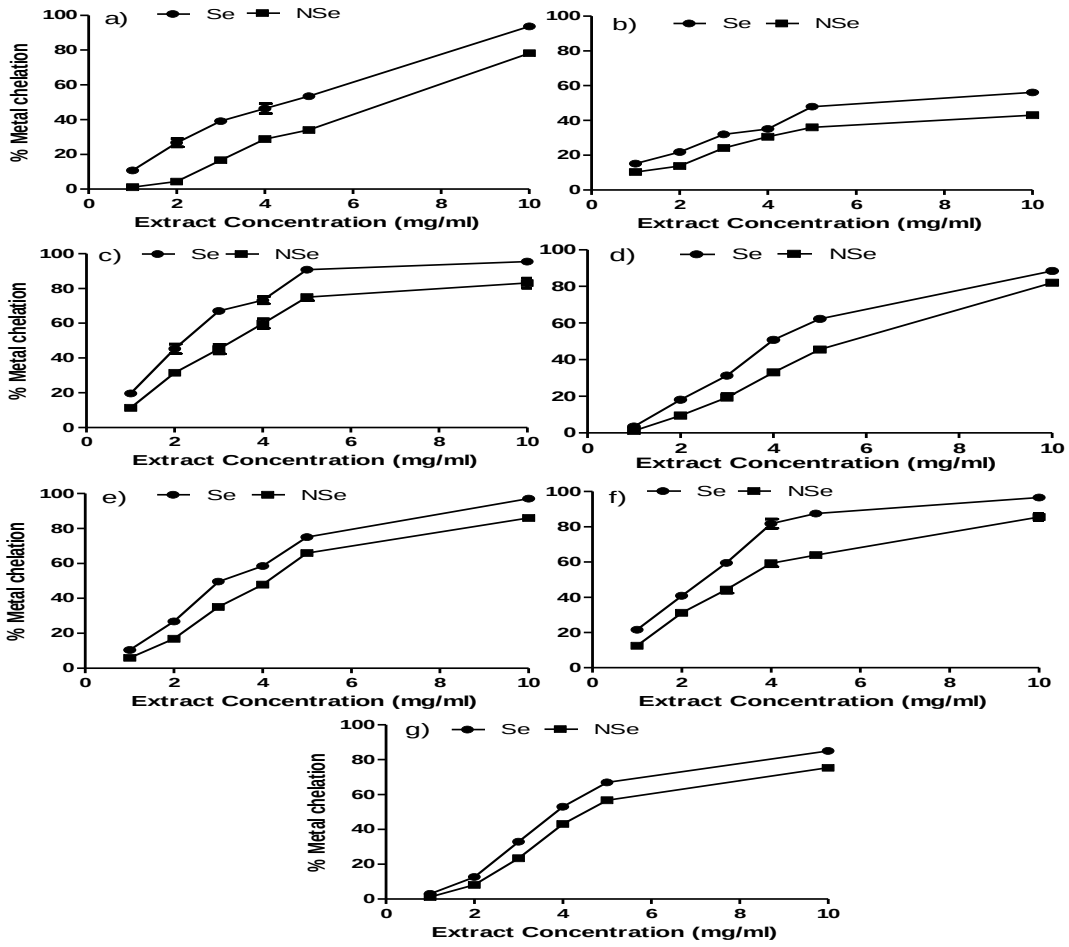


Figure 4.19. Metal chelation ability in Se-rich fruiting bodies in terms of fold increase over control (NSe)

The statistical tests of significance on metal chelation showed no significant difference at $P > 0.05$ between Se-rich species (Fig. 4.20). However, when compared between various extract concentrations it was observed that metal chelation for a particular species increased with increasing concentration at $P < 0.001$.



Two-way ANOVA between Se-rich mushrooms
Comparison between extract concentrations: Significant at P <0.001 Comparison between Se-rich species: not significant at P>0.05

Figure 4.20. Metal chelation (%) activity of methanolic extracts of Se-rich mushrooms; a) *A. bisporus,* b) *V. volvacea*, c) *P. sajorkaju*, d) *P. ostreatus*, e) *P. florida*, f) *P. djamor*, g) *P. itrinopileatus*; as compared to their respective controls (n=3)

4.5.7 Bioactivity of Se-rich mushroom extracts in terms of cytotoxicity on proliferation of Lung cancer cell lines (A549)

To examine the effect of bioactive fractions on proliferation of cancerous cells, the crude extracts of mushrooms (Se and NSe) from four different solvents viz. hexane, ethanol, methanol and water were tested on Lung cancer (A549) cell lines (Table 4.16). Se-rich bioactive extracts showed significantly higher cytotoxicity than NSe extracts against cancer cell lines. In case of *A .bisporus*, out of the four solvent fractions, hexane fraction showed significant inhibition of cell proliferation followed by ethanol, methanol and water. The Se-rich fraction inhibited the proliferation by 89.6%, 87.9%, 64.9% and 47.5% as compared to 83.2%, 42.3%, 4.4% and 9.4% inhibition by non-Se mushrooms in hexane, ethanol, methanol and water fractions respectively.

Table 4.16. Cytotoxicity (%) exhibited by Se-rich solvent fractions against A549 cancer cell lines as compared to control mushrooms (n=3)

	Cytotoxicity %			
	Solvent fraction			
Sample	**Hexane**	**Ethanol**	**Methanol**	**Water**
***A.bisporus* (Se)**	89.6 ± 0.5	87.9 ± 2.2	64.9 ± 8.0	47.5± 3.2
***A.bisporus* (NSe)**	83.2 ± 3.7	42.3 ±2.9	4.40 ± 3.9	9.4 ± 5.5
	*	***	***	***
***V. volvacea* (Se)**	74.8 ± 0.61	66.0 ± 0.19	54.0 ± 4.1	98.8 ± 0.08
***V. volvacea* (NSe)**	70.5 ± 2.41	55.0 ± 5.90	3.0± 1.20	98.7± 0.08
	*	*	***	Ns
***P. sajorkaju* (Se)**	71.6 ± 2.60	91.8 ± 0.79	88.3 ± 1.98	83.0 ± 1.12
***P. sajorkaju* (NSe)**	51.0 ± 0.64	0.0 ± 0.06	11.1 ± 3.80	55.0 ±3.90
	***	***	***	***

(*: P<0.05; ***: P<0.001; ns-non-significant)

In case of *V. volvacea*, various solvent fractions showed marginal to significant inhibition of cell proliferation. The Se-rich fraction inhibited the proliferation by 98.87%, 74.8%, 66.0%

and 54.0% as compared to 98.71%, 70.5%, 55.0% and 3.0% inhibition by non-Se mushrooms in water, hexane, ethanol and methanol fractions respectively (Fig. 4.21).

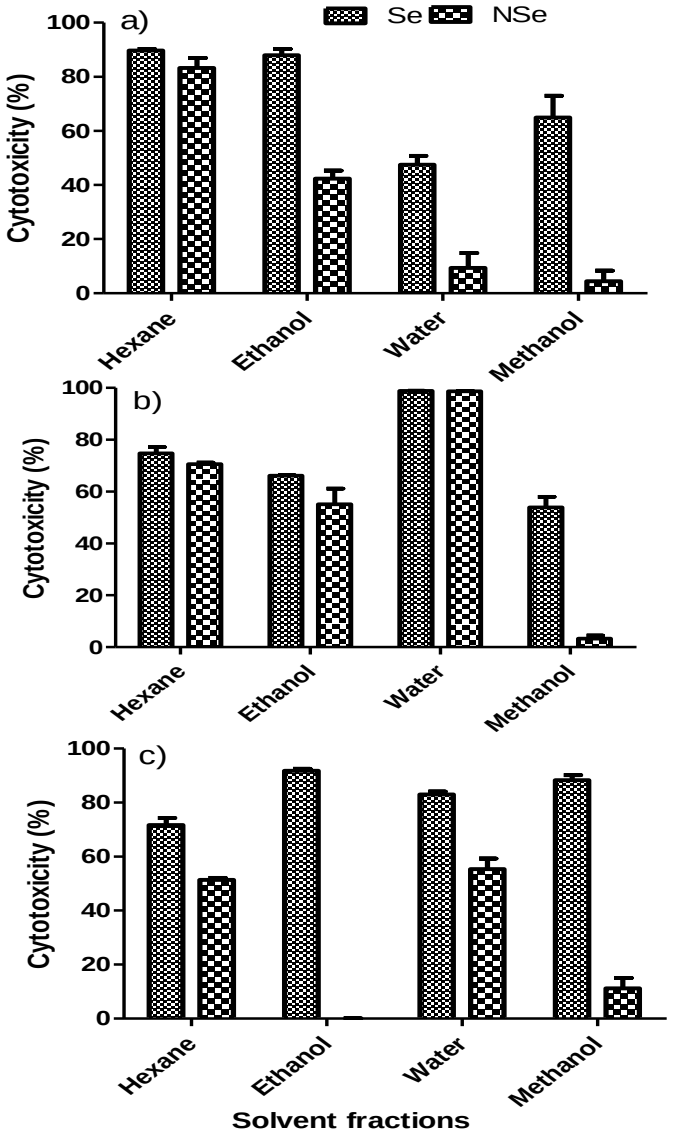


Figure. 4.21. Percent (% age) inhibition of cancer cell proliferation against A549 cancer cell lines by different solvent fraction from Se enriched mushrooms (a) *A.bisporus*;(b) *V. volvacea*; (c) *P. sajorkaju* and their respective control (n=3)

Similarly, in case of oyster mushroom (*P. sajorkaju*) ethanol fraction showed maximum inhibition of cell proliferation followed by methanol, water and hexane. The Se-rich fraction inhibited the proliferation by 91.8%, 88.3%, 83.0% and 71.6% as compared to 0%, 11.1%, 55.4% and 51.0% inhibition by non-Se mushrooms in ethanol, methanol, water and hexane fractions respectively. However, when compared at species level among the three different mushroom species; Se-rich bioactive extracts of *P. sajorkaju* showed much potential in inhibiting proliferation of cancer cells in all four fractions. It was observed that all Se-rich species exhibited almost 1 - 93 fold increase in % inhibition against cancer cell proliferation over the control species in various solvent fractions (Fig. 4.22).

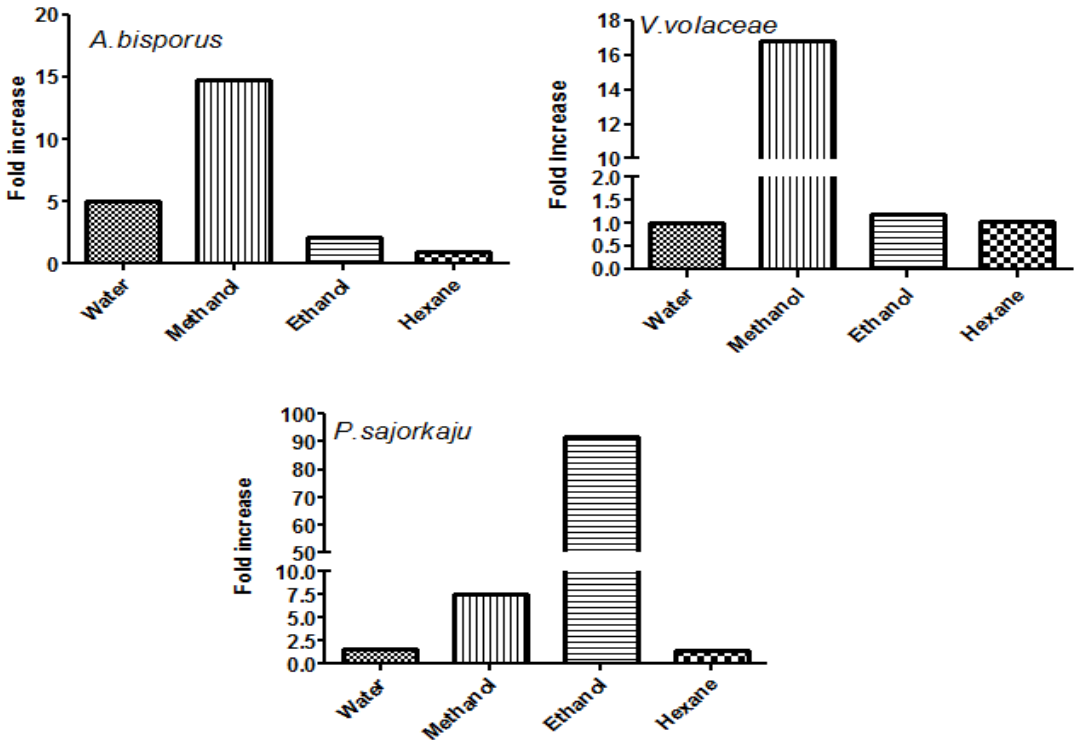


Figure: 4.22. % Cytotoxicity exhibited by Se-rich fruiting bodies extracted in different solvent fractions in terms of fold increase over control (NSe)

4.6 Preliminary observations on selenoergothioneine-like moiety

The Se-rich extracts of *A.bisporus* were analysed for possible presence of selenoergothioneine (selenium analouge of ergothioneine) like moiety expressed as a function of selenium hypaeraccumulation. A preliminary indication of presence of such moiety was reached through FTIR, wherein the Se-rich extracts showed absorption bands at 3424, 2925, 2516, 1630, 1409, 1045 and 931 cm^{-1} (Fig.4.23.). A broad band centered at 3424 cm^{-1} was assigned to hydrogen-bonded hydroxyl and amine groups. The band at around 2925 cm^{-1} was assigned to a C-H stretching vibration. The peak at 2516 cm^{-1} was assigned to an O-H stretching angle. The absorptions at 1630 cm^{-1} were assigned to the stretching vibrations of the CHO and C=O bonds. The broad absorption bands with strong intensities at 1409 cm^{-1} could be assigned to bending vibrations of O-H bond. The peak at 931 cm^{-1} indicated the existence of C=Se in the structure.

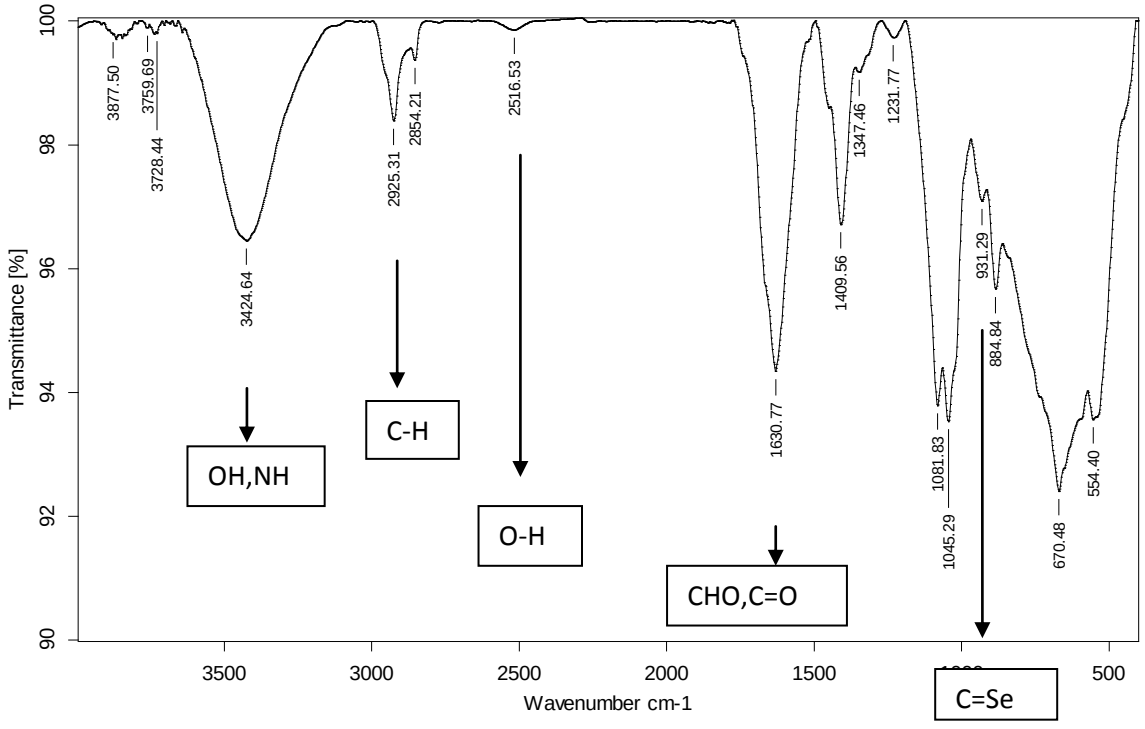


Figure: 4.23. FTIR spectra of crude extract of Se-rich mushrooms

Extending further on the preliminary studies, mass spectral analysis showed the presence of a molecular ion $[M+H]^+$ peak at m/z 278.1 (Fig. 4.24) which corresponds to the molecular formula $C_9H_{15}N_3O_2Se$ and calculated mass 277.1. This compound with the molecular formula $C_9H_{15}N_3O_2Se$ appears to be Se analogue of ergothioneine ($C_9H_{15}N_3O_2S$) where sulphur is replaced with Se (when accumulated at substantial rates).

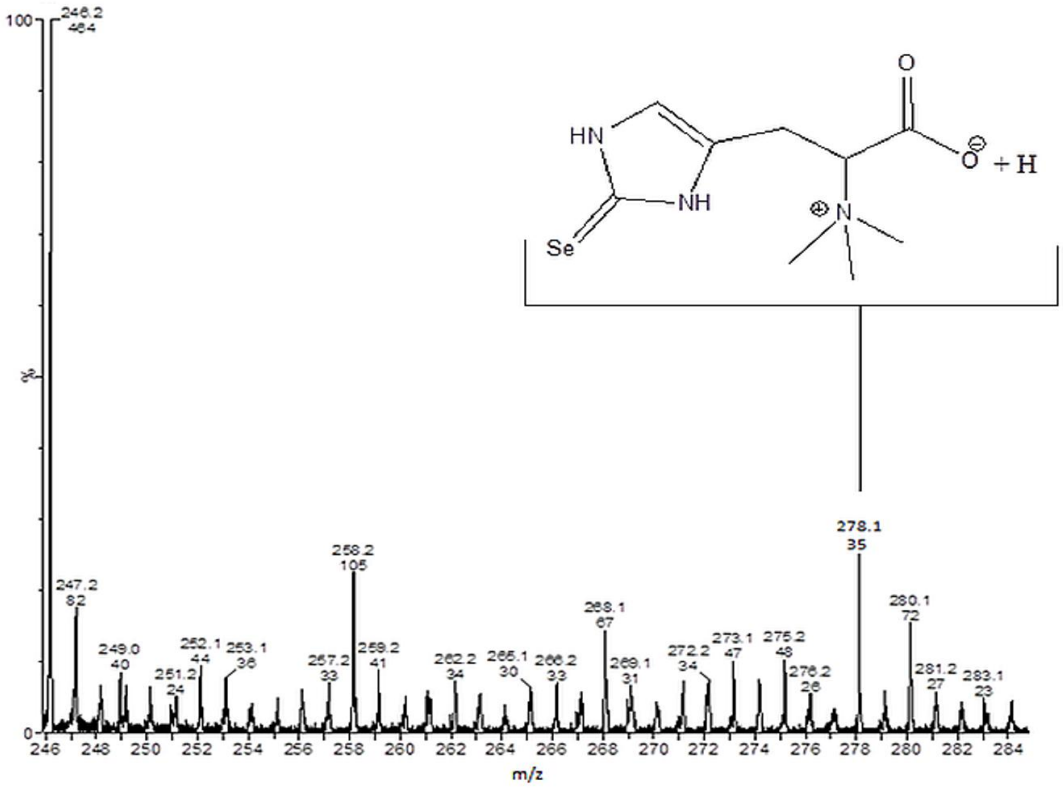


Figure 4.24. Mass spectra of crude extract from Se-rich mushroom

However, the FTIR and MS observations are only suggestive and give preliminary indication of presence of selenergothioneine like moiety in Se-rich extracts and needs furthur investigation based on isolation of pure compound from enriched mushrooms.

5. Discussion

The present study was planned to cultivate Se-rich mushrooms on agricultural wastes from seleniferous sites and to evaluate the effect of selenium on antioxidant status of the mushrooms as compared to non-enriched cultivated on normal straw. The studies conducted yielded mushrooms with substantially high selenium content and also indicated positive effect of selenium on antioxidant properties of Se-rich mushrooms as compared to non-enriched ones.

5.1 Selenium content in agriresidues

The agricultural residues belonging to wheat and paddy collected from seleniferous sites of Punjab were analyzed for total selenium content and were furthur used for cultivation of selenium enriched mushrooms. Similarly Se content in residues collected from non-seleniferous sites was also estimated and wastes were furthur processed for cultivation of non-enriched mushrooms.

Biofortification of foods through natural Se resources like agricultural residues from Se contaminated sites is an effective strategy to deal with the deficiency disorders at regions devoid of selenium. Moreover, selenium can be efficiently taken up by plants grown on seleniferous regions with elevated levels of selenium in topsoil. Most of the studies reported till date has been focused on Se fortification in mushrooms through exogenous supplementation of Se dominantly as selenite (Gergely et al., 2006). However, agricultural residues naturally enriched with Se that have significantly high Se levels can effectively be used for generating Se-enriched mushrooms. The post-harvest agricultural residues generated in seleniferous region of Punjab, India, contain significantly high Se content in plant parts such as grains and straw of wheat and other cereals, as reported by Dhillon and Dhillon, 2003a and Sharma et al. (2009). The selenium concentration in

non-enriched wheat (1.9 ± 0.8 µg Se/g DW) and paddy (2.0 ± 0.6 µg Se/g DW) substrates was found to be higher than earlier reports lying in the range of 0.09-0.19 µg Se/g DW in different substrates (Lee et al., 2009; da Silva et al., 2012). On the other hand selenium concentration in Se-rich wheat (24.0 ± 0.2) and paddy (29.7 ± 0.9) straw were within the range (11-146 µg Se/g in shoots of wheat) of concentrations reported by Cubadda et al. (2010) from identical area and higher than Se-rich substrates (Elephant grass and Rye grass) from Se-laden sites with selenium concentration of 0.8mg/kg (Lin et al., 2014). The agricultural residues collected from seleniferous sites with considerably high selenium content could therein be effectively used as prospective raw material (organic source of Se) for cultivation of Se-rich mushrooms.

5.2 Mushroom cultivation on Se-rich agricultural residues

In the present study, post harvest agricultural residues belonging to seleniferous sites were collected and used for cultivating Se-rich mushrooms. Effect of Se accumulation on growth and yield parameter of mushrooms was monitored by comparing the total yield and biological efficiency (BE) of Se-rich mushrooms with control (NSe) cultivated on non-seleniferous substrates.

The current study observed no significant difference in terms of yield and biological efficiency of Se-rich mushrooms over control mushrooms. The results are in agreement with the observations of Savic et al. (2011), reporting no negative impact of Se accumulation on growth and yield of *A. bisporus* cultivated on organic selenium source. Yield and biological efficiency of Se-rich *P. eryngii* cultivated on substrate supplemented with sodium selenite was found to be almost comparable to that of non-supplemented (Estrada et al., 2009). In contrast to organic Se-supplementation a parameter used in current study, numerous reports are available on effect of selenium supplementation mostly through inorganic forms, on growth and yield of different

matrices. Carvahlo et al. (2003), studied the effect of Se supplementation (both inorganic and organic forms) on yield in terms of fresh weight (edible portion) in tomato and strawberry and found no significant difference over control except for high organic selenium treatment. On the contrary, Werner and Beelman (2002), reported a significant negative relationship on crop yield with *A. bisporus* at higher inorganic Se levels. Similarly, a reduction of 10-15 % in yield of dill plants (Cankur et al., 2006) was observed when exposed to Se (IV) as a feature of selenium toxicity. The present study did not show any symptoms of selenium toxicity in terms of growth (total yield and BE), probably due to usage of naturally enriched substrate serving as an effective source of organic selenium.

To study the intra–species selenium interaction, comparison was drawn among different species of Se-rich mushrooms amongst which *A. bisporus* showed highest yield followed by *Pleurotus sp*. and *V. volvacea*. Cultivation of mushrooms on substrates belonging to different sources leads to different yields, due to the biological and chemical differences between them (Laborde et al., 1993, Ragunathan and Swaminathan, 2003). Mushroom cultivation is dependent upon substrate- and fungal-associated factors (Buswell et al., 1993). Substrate-associated factors are the chemical and structural properties of the lignocellulosic substrate. Fungal determinants are essentially the nutritional and environmental requirements of the cultivated strains, which are predominantly genetically determined, and the ability to produce the degradative enzymes necessary for lignocellulose bioconversion. Therefore, this difference can be attributed probably due to two reasons (i) On the substrate used to cultivate mushroom (ii) the tendency of particular organism to uptake and accumulate different chemical forms of selenium from the respective substrate.

5.3 Selenium uptake and accumulation in mushrooms

This section of study was planned to analyze the selenium accumulation potential of edible mushrooms belonging to three different genera. The results obtained revealed that all seven species studied had substantially high selenium accumulating potential from the substrates used for cultivation.

The natural and commonly processed food sources are generally found less abundant in selenium (Spallholz et al., 2004), therefore there is a utmost need of Se-enriched food sources for adequate supplementation. For the same purpose, a number of selenium enriched dietary matrices studied, which include brazil nuts (Vonderheide et al., 2002), yeast (Mcsheehy et al., 2005), green tea (Xu et al., 2007), some of selenized vegetables like brocolli (Pedrero et al., 2007), garlic (Dong et al., 2001), onion (Tejo Prakash et al., 2010; Sharma et al., 2007), and mushrooms (Maseko et al., 2013; Ogra et al., 2004; Stefanka et al., 2001). Mushrooms are considered valued products rich in mineral compounds. Mushrooms are generally grouped with vegetables for dietary purposes, but are not considered as plants. Selenium content of mushrooms is generally higher than that of most vegetables (Rayman et al., 2008) but it is highly variable. A wide number of mushrooms have been studied for uptake of selenium, but species vary in the selenium content with respect to each other. In general mushrooms have been found to contain selenium < 1 mg/kg DW (Falandysz, 2008). Many attempts have been made to grow Se-rich mushrooms through artificial means, however, less attempts are reported for cultivation through naturally enriched matrices.

The resulting concentrations in fruiting bodies of *Pleurotus* and *Volvariella* mushrooms cultivated on unenriched substrates was in range of 2.97 - 6.0 μg Se/g DW which is quite comparable to the typical range (0.2 – 5.0 μg Se/g DW) of various wild and cultivated

mushrooms belonging to *Pleurotus* and *Agaricus* species (Anderson et al., 1982; Spolar et al., 1999; Vetter et al., 2005; Falandysz, 2008; Costa-Silva et al., 2011; Savic et al., 2011; Hong et al., 2011; da Silva et al., 2012) and comparatively less than *A. bisporus* with selenium concentration of 14.42 μg Se/g DW. This is quite similar to selenium concentration of *A. bisporus* (cultivated on unenriched compost) with selenium content of 12.2 μg Se/g DW in dried caps (Maseko et al., 2013) and much lower than wild edible species of *Boletus edulis* and *Albatrellus pes-caprae* with selenium concentration in the range of 20-70 and 200 μg Se/g DW (Falandysz, 2008).

All seven species of Se-rich mushrooms were able to mobilize and accumulate significant amount of selenium from their respective substrates naturally rich in selenium over control mushrooms cultivated on control substrates. The ability of other mushroom species to absorb inorganic Se from the growth substrate present in the range of 5-102 μg Se/g and accumulate Se in the mycelium as well as in the fruiting bodies (in the range > 1.5-858 μg Se/g DW) has been reported by various research groups (Wang et al., 2005; Estrada et al., 2009; Cremades et al., 2012; da Silva et al., 2012). Similar observations have been found to support the current results wherein Se-rich fruiting bodies cultivated on substrates supplemented with organic selenium were found to contain considerably higher selenium concentration over control mushrooms (Gergely et al., 2006; Savic et al., 2011; Savic et al., 2012)

Se-rich fruiting bodies belonging to *Pleurotus*, *Agaricus* and *Volvariella* species accumulated selenium in range of 26.1 - 145.4 μg Se/g DW which lies in typical variable range (3.12 - 137.84 μg Se/g DW) of Se-rich mushrooms (Estrada et al., 2009; Cremades et al., 2012; Savic et al., 2012; da Silva et al., 2012) and comparatively lower than Se concentrations observed in mycelia or fruiting bodies of *Trametes versicolor* (600-1500 μg Se/g DW), *Agaricus*

bisporus (415.0 - 770.7 µg Se/g DW) and *Lentinula edodes* (748.0 µg Se/g DW) grown on substrates supplemented with different concentrations of inorganic and organic forms of selenium (Gergely et al., 2006; Tham et al., 2009; Turlo et al., 2010; Maseko et al., 2013). High variation in terms of selenium concentration was observed among all Se-rich species for example when species of three different genera were compared the order of increase was *Pleurotus* > *Agaricus* > *Volvariella*. Bioaccumulation of metalloid like selenium by fruiting bodies of different mushrooms is species specific. Lavola et al. (2011), reported high variation in accumulation of boron between various species of ectomycorrhizal and saprophytic fungi when grown on same forest soil with common boron content. Similar kind of observation has been reported in a store of North America from where two brands of the same corn product were purchased and were found to have 10 fold differences in selenium concentrations (Finley et al., 1996). Same pattern was followed in present study, where mushrooms of different genera/species grown on wheat straw with identical selenium concentration yielded variable selenium content. Species varied drastically in terms of accumulation over each other. Other important factors are the bioavailable Se content of soil (Wu et al., 2003; Goh and Lim, 2004), growing conditions such as substrate composition and flush cycle, therefore the selenium content of identical products can be highly variable.

Expressed on a fresh weight basis, the Se concentration, obtained in the present study, was 12.0, 14.5, 11.0, 4.3, 4.4, 2.6 and 3.5 µg Se/g FW in Se-fortified fruiting bodies of *A. bisporus, P. djamor, P. florida, P. sajorkaju, P.ostreatus, P. citrinopileatus* and *V. volvacea* respectively. Assuming negligible losses due to cooking, consumption of 4.5 g (*A. bisporus*), 3.8 g (*P. djamor*), 5.0 g (*P. florida*), 12.7 g (*P. sajorkaju*), 12.5 g (*P. ostreatus*), 21.0 g (*P. citrinopileatus*) and 15.7 g (*V. volvacea*) of mushrooms is sufficient to meet the recommended

dietary allowance (RDA) for adults, which is set to 55 μg Se/day (Bhatia et al., 2013; IOM, 2000). The RDA obtained in the present study for *A. bisporus* on fresh weight basis (4.5 g) was found to be much lesser in terms of consumption as compared to RDA (180g) recommended by Cremades et al. (2012) for Se-fortified *A. bisporus*. The current study proposes that Se-rich mushrooms cultivated on agricultural residues collected from seleniferous sites can serve as prospective dietary source of organic selenium than those supplemented with inorganic sources exogenously, as the form and concentration of selenium source. Our interpretation is well supported by the conclusions of Dernovics et al. (2002), which found organic source of selenium in the form of selenized yeast to be more bioavailable than Na_2SeO_3 for absorption and accumulation.

5.4 Selenium bioaccessibility in mushroom

Knowledge of speciation and bioaccessibility of an element is essential to evaluate a food as a potential dietary source of that particular element. For full expression of selenoproteins in terms of antioxidant functioning, a good bioavailable source of selenium from dietary sources is required to deal with problems related to oxidative stress. Accumulation of selenium by fruiting bodies of Se-enriched mushrooms grown on substrates supplemented by inorganic/organic sources and expression of results as total selenium content has been already reported (Vetter and Lelley, 2004; da Silva et al., 2012). Determination of total selenium content is although very important to know the elemental mass balance, yet total mineral content alone cannot provide sufficient information regarding metabolism and physiological needs, hence it must be accompanied by speciation and bioaccessibility studies (Uden, 2002). There is a definite need of searching selenium species other than total mineral content as different ingested selenium species follow different metabolic pathways in the body (Letavayova et al., 2006).

Keeping the above mind, apart from studying total selenium content, speciation and bioaccessibilty of selenium species liberated by gastrointestinal hydrolysates (GI) of Se-rich *P. florida* was also monitored using size exclusion chromatography (SEC) and anion exchange chromatography coupled to HPLC-ICP-MS.

For separations of Se-containing species in liquids, high-performance liquid chromatography (HPLC) at anion exchange columns (Stadlober et al., 2001) reversed-phase columns (Mcsheehy et al., 2000), or size exclusion columns (Ferrarello et al., 2002), have been successfully coupled with element sensitive detectors, such as ICP–MS. Selenium in food as well as biological samples mainly occurs in the form of non-volatile compounds like selenoaminoacids, inorganic species and polypeptides. As HPLC-ICP-MS is much more accurate in terms of sensitivity as compared to other techniques, it has been applied to detect various non-volatile selenocompounds of high and low molecular weight in the from of SeMet in *Lentinula edodes*; Se-methyl selenocysteine and SeMet in *Allium sativum* and *Brassica juncea* respectively (Ogra et al., 2004; Montes-Bayon et al., 2006). Selenium speciation has already been reported by various researchers in different Se-enriched mushrooms with detection of selenocompounds such as SeMet (Ogra et al., 2004; Gergely et al., 2006), SeCyst (Yoshida et al., 2005) and MeSeCys (Gergely et al., 2006). In this study, the GI extracts subjected to SEC-HPLC-ICP-MS yielded peptides and low molecular weight selenocompounds of weight < 5 KDa. The main compound detected was selenomethioneine (SeMet) along with another major peak that corresponds to SeMet-selenoxide (SeOMet) and Se-peptides unspecifically produced by the gastrointestinal juice. Non-solubilized species of selenium liberated after GI digestion might be present in form of undigestible Se-containing polysaccharides. It has been reported by Munoz et al. (2006) that part of the Se in Se-enriched mycelia of *P. ostreatus* is associated with chitin-containing

structures in cell walls. Formation of Se-containing polysaccharides might be due to the low Se bioavailability found elsewhere for other species (Chansler et al., 1986; Mutanen, 1986). Similarly, limited bioavailability might be the result of a low bioaccessibility due to a larger incorporation of Se in mushroom polysaccharides compared to *P. florida* in the conditions of the present study.

Our results are in accordance with the observations of Diaz-Huerta et al. (2005), wherein SeMet was found to be the dominant bioavailable form of selenium along with presence of some unknown species of low molecular weight in water extracts of fruiting bodies of three edible mushrooms (*Macrolepiota procera*, *Lepista luscina* and *Buletus luridus*) analyzed by SEC-ICP-MS. Similarly, in another study, conducted by many separation techniques such as SEC-ICP-MS and ion exchange chromatography, Se-accumulating mushrooms (*Albatrellus pescaprae* and *Boletus edulis*), dominantly contained low molecular weight compounds (6 KDa) with a small fractions of selenite, SeCyst and SeMet and a bulk of compounds left unidentified (Slejkovec et al., 2000). Gergely et al. (2006), reported presence of SeCyst along with presence of SeMet, methyl selenocysteine and inorganic forms in *A. bisporus* and *Lentinula edodes* by means of SEC-ICP-MS. The extracts of *Boletus* mushroom contained Se primarily associated with a fraction between 2.9–3.2 KDa and a low-molecular-weight fraction along with that 10 % of Se in the NaOH extraction with a fraction at 50 KDa (Wuilloud et al., 2004).

After first fractionation of GI by SEC-ICP-MS, the collected fraction was subjected to anion exchange chromatography coupled to HPLC-ICP-MS to furthur characterize the individual selenium species. The dominant form of selenium was found to be SeMet which accounted for 73% of sum of species detected along with minor concentration of SeOMet and Se (IV) and about 25% of unidentified species. Similarly the aqueous extracts of shitake mushroom

accounted for 68% of total selenium in extracts liberated after simulated cooking process, with SeMet as the major bioavailable form as analyzed by LC-ICP-MS and LC-ESI-MS (Ogra et al., 2004). Similar results has been reported by Hinojosa et al. (2006), wherein Se-rich yeast subjected to *in-vitro* gastrointestinal digestion yielded 90% of total selenium to be in bioavaliable form and SeMet accounting for 40 % content out of total bioavailable forms.

Se-biofortified *P. florida* mainly constituted organoselenium compounds, with SeMet as the major species, therefore indicating its high nutritional potential as compared to selenized mushrooms accumulating primarily inorganic Se (Estrada et al., 2009; da Silva et al., 2012; Cremades et al., 2012; Finley, 2006; Bhatia et al., 2013). There is good evidence that increased Se status attained after supplementation with organic forms of Se is retained for a longer period after supplementation has ceased than is the case with Se (IV) or Se (VI) (Rayman et al., 2008). Therefore Se-fortified *P. florida* can serve as a prospective dietary source of organic selenium with better bioaccessibilty over extended time. The present study illustrates the feasibility of generation of Se-fortified mushrooms through cultivation on agricultural wastes collected from seleniferous sites. These dietary matrices can therefore serve as important source of organic selenium depending on its concentration and speciation in mushrooms.

5.5 Elemental and protein profile in mushrooms

5.5.1 Elemental composition

Mushrooms are widely appreciated across world due to their high nutritional status; therefore it's necessary to study their nutritional composition in terms of essential elements. The minor and essential elements are of great biochemical importance and have nutritional and clinical values. Macro-fungi possess variable and highly specific ability for uptake of various

trace elements (metals, metalloids) from soil and subsequent accumulation in fruiting bodies. Metals such as Mg, Ca, Na, P and Zn are considered to be vital ions in body and are important co-factors of various enzymes (Higaki et al., 1992; Regan, 1993; Griffith, 1995).

The present study was conducted to analyze and compare the nutritional status of Se-rich mushrooms (in terms of elemental profile) with control species. Quantification of five different elements i.e. iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg) and calcium (Ca) was carried out in fruiting bodies of Se-rich and control mushrooms. The ability of mushroom species to bioaccumulate the mineral forms from the growth substrate to fruiting is well documented (Rajarathnam et al., 1998; Kalac, 2010). All seven species of Se-rich and control mushrooms absorbed substantial amount of metals analyzed during study with no significant difference between them in relation to uptake. The order of accumulation of elements in current study was Mg > Ca > Fe > Zn > Cu. The concentration of Mg, Ca, Fe, Zn and Cu metals in Se-rich and control mushrooms was found to be in range of 0.92 - 1.99; 0.16 - 0.86; 0.1 - 0.52; 0.07 - 0.26 and 0.02 – 0.13 mg/g DW respectively, which are quite comparable for Mg (1.1 - 1.3 mg/g DW), Cu (0.01 - 0.05 mg/g DW) and Ca (0.19 – 0.86 mg/g DW) studied in *A. bisporus* and *Pleurotus* species (Manzi et al 1999; Vetter, 2003; Vetter et al., 2005) and higher than elemental range reported for Mg (0.002 – 0.35 mg/g DW); Ca (0.001 – 0.34 mg/g DW); Fe (0.001 – 0.55 mg/g DW) and Zn (0.0004 – 0.26 mg/g DW) in fruiting bodies of *Volvariella* and *Pleurotus* species (Alam et al., 2008; Mallikarjuna et al., 2013). The uptake and corresponding concentration of metal ions by macro-fungus dominantly depend on the mushroom species and their ecosystem (Gast et al., 1988).

The present study indicated that Se-rich mushrooms showed almost comparable uptake of all elements with respect to control mushrooms. Selenium enrichment did not alter the nutritional

status/elemental composition of mushrooms. Our observations are in accordance with the observations of da Silva et al. (2012), wherein selenium addition to substrate did not affect the overall elemental content of mushrooms.

5.5.2 Total protein content

Since long back, mushrooms have been globally well accepted due to their flavour and texture as vegetables. They are found to be very rich sources of protein, fibre vitamin and minerals (Manzi et al., 1999; Matilla et al., 2000; Agrahar and Subbulakshmi, 2005; Kalac, 2009). Mushrooms contain ~ 22% protein containing essential amino acids (Matilla et al., 2000). Mushrooms belonging to both Se and control species exhibited high protein content lying in the range of 274 – 316 mg/g on dry weight basis. The observed values were comparable to the crude protein content (243.2 – 372.8 mg/g DW) estimated in different wild grown mushrooms (Heleno et al., 2011; Akata et al., 2012;) and were higher (70.2 – 239.1 mg/g DW) than those observed in different species of cultivated mushrooms (Dundlar et al., 2008; Alam et al., 2008; Lee et al., 2009; Ulziijargal and Mau, 2011; Jaworska et al., 2011; Reis et al., 2012). A significant difference between all Se-rich species over control was observed when comparison was drawn for relative protein content in fruiting bodies. Similar observation was reported by Maseko et al. (2013), wherein total protein content in Se-fortified fruiting bodies of *A. bisporus* increased with increasing concentration of selenium in substrate and was found to be significantly higher than control. On the contrary, no significant difference in total protein content was observed in fruiting bodies of *A. bisporus* cultivated on Se-rich and standard compost (Cremades et al., 2012). Among various Se-rich species, the protein content varied depending upon the selenium concentration and was found to be highest for *A. bisporus* (316.0 mg/g DW) followed by *Pleurotus* species (292.7 – 314 mg/g DW) and *V. volvacea* (301.1 mg/g DW). Since mushrooms

contained high protein content, therefore it is reasonable to anticipate that selenium may get incorporated into protein when it is accumulated at high levels, a feature observed in the present case. Since Se-rich mushrooms contained significant amount of protein on dry weight basis, it is quite acceptable that mushroom protein fractions may contain high levels of organic selenium. This is supported by observations in *P. florida*, wherein the GI extracts subjected to SEC-HPLC-ICP-MS yielded peptides and low molecular weight selenocompounds dominantly present as selenomethioneine (Bhatia et al., 2013). Therefore, the consumption of Se-rich mushrooms cultivated on selenium rich substrates (naturally enriched) with abundant protein fractions containing organic selenium can serve as good dietary food, with appropriate formulation of a well balanced diet dealing with both nutritional as well as functional attributes.

5.6 Antioxidant properties of mushrooms

5.6.1 Extraction yield

Sample extraction is necessary to convert the samples to the forms that can be analyzed with due respect to bioactive properties of interest. In the present study, yield of methanolic extracts of seven species were analyzed to check their extraction efficacy. Extraction yield of methanolic extracts belonging to Se-rich and control mushrooms was found to be reasonably high (28.0 - 45.0 %) for all species. These observations were in accordance with the extraction yield (15.9 - 43.9 %) of methanol extracts of fruiting bodies belonging to *Flammulina*, *Lentinula*, *Volvariella* and *Pleurotus* species (Mau et al., 2002; Yang et al., 2002; Cheung and Cheung, 2005; Oke and Aslim, 2011). Selenium rich methanolic extracts yielded significantly higher extraction yield when compared to control extracts. Our results are in contrast to the observations of Turlo et al. (2010), wherein Se-rich mycelium as well as fruiting showed comparatively lower

extraction yield than their control, which could be attributed to the source of selenium enrichment used during the study i.e., inorganic forms that may cause toxicity to the organism at certain point. However, in present study, mushrooms accumulated selenium from natural sources dominantly containing organic moieties of selenium, which could be the probable reason for higher extraction yield. Apart from these, there are many factors that affect the extraction efficacy such as nature of sample, extraction conditions etc. Among different Se-rich species the extraction yield varied significantly which might be a species specific modulation. Difference in yield can also be credited to the extractibility of different components with varying polarity of solvent system.

The current results indicated that most of the components in 50 % methanol were high in polarity and extracted efficiently thereby indicating possible enhancement in biological efficacy of Se rich mushrooms to be used for various antioxidant studies.

5.6.2 Total phenol and total antioxidant content

Polyphenols are although known to be non-nutritive compounds yet they are reported to have specific health effects, such as antioxidant properties of phenols play a vital role in antioxidant mechanism and stability of food products (Wright et al., 2001). Mushrooms can accumulate a variety of secondary metabolites such as phenolic compounds which are reported to be excellent antioxidant, antitumour and anti-inflammatory agents (Puttaraju et al., 2006). Mushrooms are rich source of polyphenols and are widely evaluated for the same by several authors (Ferreira et al., 2007; Barros et al., 2007; 2008;)

Total phenolics are the most dominantly occurring natural antioxidant moieties extracted in methanolic extracts of several mushroom species (Mau et al., 2004; 2005). The antioxidant

activity of mushroom extracts is commonly correlated with the total phenol content, evaluated through colorimetric methods such as Folin-Ciocalteau assay, therefore Se-rich extracts belonging to different mushrooms species were studied for total phenol content to analyze the fate of phenol content in presence of selenium. In general, all mushroom extracts belonging to both Se-rich and control species were found to be excellent sources of phenol with levels lying in the range of 3.15 – 17.7 mg GAE/g DW. Our studies were comparable with the total phenol content (2.6 – 15.0 mg GAE/g DW) estimated by other research groups in methanolic extracts of different mushroom species (Cheung et al., 2003; Puttaraju et al., 2006; Palacios et al., 2011; Hung and Nhi, 2012; Reis et al., 2012), except for Se-rich *V. volvacea* which showed higher phenol content (17.7 mg GAE/g DW) than range observed. However, the levels observed were lower in comparison to total phenol (23.3 mg GAE/g DW) estimated in methanolic extracts of *A. bisporus* by Reis et al. (2012). Se-rich mushrooms showed significantly higher phenol content over control across all species studied, with *V.volvacea* exhibiting highest content and *A. bisporus* being the lowest. It may be due to inhibition of enzymatic polyphenol oxidation by secondary effect mediated through strong antioxidant-active selenium compounds. Our results are fully supported by observations of Turlo et al. (2010), wherein total phenol content of methanolic mycelial extracts of *Lentinula edodes* were found to be higher for selenated fractions as compared to non-selenated fractions. Similarly, total phenol content increased with rising concentration of selenium in rye crop as compared to the control (Antoneko et al., 2013). In contrast to these observations, Se-rich *A. bisporus* showed almost similar content of phenols when compared to their respective control (Cremades et al., 2012). Antioxidant activities of phenolic components play a vital role in the antioxidative defense mechanisms exhibited by the biological systems (Macheix and Fleuriet, 1998). In addition, Se is well known for its association

with antioxidant mechanisms in humans and animals. Therefore, increase in the phenol content in Se-enriched mushrooms may be due to the influence of Se in promoting the synthesis and activity of antioxidant metabolites, a feature that could be observed in the present study. Cartes et al. (2005) suggested that Se induces the antioxidant capacity by its relationship with glutatione peroxidase (GPx), the activity of which intensifies at higher Se concentrations. Activities of several enzymes such as glutathione peroxidase, glutathione reductase and superoxide dismutase are positively influenced by different classes of polyphenols (Rodrigo et al., 2005).

Total antioxidant content in Se-rich fruiting bodies followed the same trend as total phenol content. Amongst all species, *P. djamor* showed the highest antioxidant content and *V. volvacea* showed the lowest. Similarly, observations reported by Imran et al. (2011), wherein *P. eous* (Pink mushroom) showed higher activity in terms of total antioxidant content than other species studied (*P. florida*). All Se-rich mushrooms exhibited significantly higher antioxidant content than their respective controls. Similar to our observation, Rios et al. (2008) reported increase in various antioxidants in broccoli as a result of selenium application in the form of selenate. Therefore increase in total antioxidant content as a function of Se uptake, adds on to the overall antioxidant status of mushrooms.

It is also evident from the studies that phenol content affects the GPx activity which is furthur dependent on selenium (acting as an co-factor) concentration of matrices, therefore Se-rich mushrooms with high phenol and total antioxidant profile than normal mushrooms may enhance the GPx activity, thereby intensifying the total antioxidant status.

5.6.3 Inhibition of lipid peroxidation

Lipid peroxidation leads to structural and functional damage of the biomolecules along with cellular structure of cellular components; moreover oxidation of low density

lipopolysaccharides leads to clinical conditions such as artherosclerosis. Mushrooms generate phenolic antioxidants that inhibit LPO (Yen and Chen, 1995). Till date, various studies showing low to high antioxidant activities in terms of LPO inhibition has been conducted on different species of mushrooms, wherein mushrooms belonging to *Agaricus bisporus*, *Agaricus bransiliensis*, *Ganoderma lucidium* inhibited LPO by 46.3-83.4% (Kozarrki et al., 2011); ear mushrooms inhibited LPO upto 38.6-74.6% (Mau et al., 2001); some wild species of mushrooms (*Boletus edulis, Cantharellus cibarius, Craterellus cornucopiodes, Calocybe gambosa, Hygrosphorus marzuolus* and *Lactarius deliciosus*) inhibited LPO by 36.0 – 74% (Palacios et al., 2011); on the other hand *Antrodia camphorata* (Huang, 2000) and *Ganoderma lucidium* (Lin, 1999) showed excellent activity as evident by inhibition of LPO by 5.88 - 6.41 %. Apart from mushroom extracts, the antioxidant properties are also derived from essential elements like selenium which is known to have important role in LPO prevention (El-Demerdash, 2004). The current study was carried out to visualize the effect of selenium on lipid peroxidation profile of mushrooms. Se-rich mushrooms belonging to all seven species exhibited significantly lower MDA levels (a measure of LPO) than their respective controls. This may be due to rich antioxidant profile of Se-rich mushrooms in terms of high phenol and total antioxidant content as discussed in earlier sections. This statement is supported by the fact that phenolic compounds exhibit antioxidant activity through inhibition of oxidation of low density lipoproteins (Vinson et al., 1995; Teissedre and Waterhouse, 2000). Cheung et al. (2003) also correlated the antioxidant activities of mushrooms with their phenolic content. Our results are supported by observations of Turlo et al. (2012), wherein Se-rich methanolic mycelial extracts showed significantly lower lipid peroxidation than control extracts. The same group suggested that high concentration of selenium in mycelial extracts leads to more pronounced antioxidant properties such as LPO and

scavenging activities than control. Song et al. (2009) reported that Se-enriched mycelial extracts of *Stropharia rogoso-annulata* exhibited relatively lower MDA levels than other treatments. Similarly, Tejo Prakash et al. (2010), observed a positive correlation between selenium concentration and antioxidant activities in *Allium cepa*. Selenium role in inducing antioxidant capacity in terms of decrease in lipid peroxidation has already been reported by Fatma and Demerdash (2004), wherein selenium supplementation reducing the level of free radicals by decreasing total lipid content and enhanced activity of glutathione S transferase. Similarly in another study, selenium supplementation prevented LPO in brain tissues of rats supplemented with 0.6 mg/kg of sodium selenite as compared to control, when subjected to arduous exercise (Akil et al., 2011).

Apart from these studies, many researchers have studied the effect of supplementation of selenium on lipid peroxidation under *in - vivo* conditions. *Pleurotus ostreatus* enriched with selenium and zinc when administered to mice, resulted in significant decrease in MDA levels as compared to those fed on normal diet (Yan and Chang, 2012). Trbojevic et al. (2010), reported significantly lower LPO in mice injected with sodium selenite than other treaments devoid of selenium. Similarly in another study, supplementation of selenomethioneine to goats lead to significant decrease in MDA values at increasing concentrations of selenium (Yue et al., 2009).

The present study, thus proposes, a possible exploration on consumption of Se-rich fruiting bodies of mushrooms cultivated over normal mushrooms to protect against oxidation of polyunsaturated fatty acids in low density lipoproteins especially in human beings due to their potential ability to reduce lipid peroxidation.

5.6.4 Free radical scavenging activity of mushroom extracts

This study, Se-enriched mushrooms were studied for antioxidant activities in terms of free radical scavenging (FRS) in comparison to regular mushrooms to understand the modulations induced by selenium. The most widely used method among free radical scavenging assays (FRS) is the DPPH scavenging model for evaluating FRS abilities of antioxidants. In this particular assay, antioxidants, with the ability to donate hydrogen, reduce the stable DPPH (purple) to the non-radical form DPPH-H (yellow). Free radicals generated due to oxidative processes pose a threat to structures and functions of cells. To deal with oxidative stress, three mechanisms operate in cells that (Bartosz, 1995) include prevention of reactions of reactive oxygen species with biologically-significant compounds; breaking free radical chain reactions and undesirable non-radical oxidation reactions; scavenging the products of free radicals reactions and repair of damages.

The free radical scavenging potential of mushroom extracts was studied by examining the effect of methanolic extract on scavenging of DPPH. Antioxidant activity in terms of free radical scavenging activity of non enriched mushroom extracts is well reported (Puttaraju et al., 2006; Kim et al., 2008). The scavenging effect of methanolic extract of Se-enriched and control mushrooms increased with increasing concentration of extract. The DPPH scavenging ability (EC_{50} mg/ml) of extracts of various mushrooms has been reported to be lying in the range of 0.9 – 6.54 mg/ml (Oke and Aslim, 2011; Reis et al., 2012); which are found to be higher than EC_{50} studied in Se-rich *A. bisporus* (0.056 mg/ml) comparable to EC_{50} value observed for Se-rich *V .volvacea* (6.0 mg/ml) and much lower than EC_{50} values for Se-rich *Pleurotus* species (9.3 – 12.4 mg/ml). The percent FRS studied in present case was found to be much higher than methanolic extracts of *Lentinula edodes* (29.4% at 9 mg/ml) reported by Cheung et al. (2003). The Se-rich

mushroom extracts showed significantly higher FRS than respective controls, with the order of scavenging found to be *A. bisporus* > *Pleurotus sp* > *V. volvacea*. The relatively high FRS activity of Se-rich mushroms over control might be due to presence of organic moieties of selenium that are known to be linked to beneficial biological effects and disease prevention (Clark et al., 1996; Flores-Mateo et al., 2006). The antioxidant effect of selenium comes from the fact that this element is an intergral part of active site of various enzymes (Glutathione peroxidase and thioredoxin reductase) responsible for protecting cells from oxidative damage arising from free radical damage (Reeves and Hoffmann, 2001). Our results are supported by the observation of Turlo et al. (2010), wherein the FRS effect of Se-rich methanol extracts was almost two times higher when compared to their corresponding controls. Similarly, protein extracts from Se-rich *Ganoderma lucidium* exhibited higher scavenging activities on superoxide and hydroxyl radicles than their regular analogs as controls (Zhao et al., 2004).

Now-a-days, a variety of antioxidants synthesized synthetically [(butylated hydroxy anisone (BHA), gallic acid and butylated hydroxy toluene (BHT)] are avaliable but has been suspected to promote negative health effects (Barlow, 1990). Therefore, there is a definite need to replace them with naturally occuring antioxidants. Mushrooms enriched with essential antioxidant elements like selenium will definitely serve as promising defense against pro-oxidant conditions, as many low molecular weight compounds containing selenium has been to exhibit antioxidant and antitumour activities (Tapiero et al., 2003).

5.6.5 Metal chelation activity of mushroom extracts

Free radical generation and their subsequent oxidative damage are well controlled by metal ion chelating properties of an antioxidant molecule. Lipid peroxidation through subsequent chain reaction initiated by metal ions is the main factor for degradation of food (Gordon, 1990).

The incidences of cancer and arthritis are also correlated with the catalysis of metal ions (Halliwell et al., 1995). Metal ion chelating reduces the concentration of the transition metal catalyzing the process of lipid peroxidation and thereby plays an important antioxidant mechanism (Dodig and Cepelak, 2004). Since ferrous ions are the most efficient prooxidants in the food systems (Yamaguchi et al., 1988), the enhanced chelating effects of methanol extracts from Se-enriched mushrooms would be more desirable over those obtained in control mushrooms. In present study, it was observed that methanolic extracts of all mushroom species exhibited low to high metal chelation effect lying in the range of 43.0 – 97.1 % at a concentration of 10 mg/ml. The studied values were found to be lower than those of *Amantia caesarea* and *Leucoagaricus pudicus* showing metal chelation of more than 90 % at 4 mg/ml (Sarikurkcu et al., 2010) and higher than observations of Lin, (1999); exhibiting chelating ability of 46.4 – 52.0 % for various mushrooms such as black stinkhorn, white matsutake and lion's mane at concentration of 24 mg/ml. Chelating effect of selenium rich mushrooms from Se-rich mushrooms was found to be significantly higher than non-enriched mushrooms. High variation in terms of metal chelation was observed among all Se-rich species when species of three different genera were compared with order of increase being *Pleurotus* > *Agaricus* > *Volvariella*. The high rate of metal chelation of Se-rich mushrooms might be due to presence of organoselenium moieties which are found to be more bioavaliable to induce selenoenzyme expression. Similarly, Turlo et al. (2010), suggested that selenium enrichment enhanced the antioxidant activities of mycelia extracts likely due to presence of high amount of organoselenium compounds.

It has been reported that metal-antioxidant coordination leads to antioxidant activity and are supported by *in vitro* studies investigating metal-mediated oxidative stress and disease. Iron-

mediated DNA damage inhibition is seen for methyl-selenocysteine, selenocystamine, 3,3-diselenobispropionic acid, and 3,3-selenobispropionic acid but to a lesser extent than with copper (Battin et al., 2006). Metal-selenium complexation may afford cellular protection against oxidative DNA damage by altering the redox potential of the metal ion and preventing reduction of H_2O_2 and thus oxidative DNA damage.

The results suggested that moderate ferrous-ion chelating ability showed by Se-enriched mushroom extracts could be beneficial to health. The high metal chelating activity of Se-enriched mushroom is therefore assumed to be due to specific metal-selenium compound interactions that greatly affect antioxidant activity based on the metal type and the special features of the Se compound.

5.6.6 Antiproliferative activity of Se-rich extracts against A549 lung cancer cell line

Several animal and human experimental studies have shown the anti-cancer effect of organic selenium in the form of selenized yeast (Spolar, 1999; Hu et al., 2010), selenized milk (Hu et al., 2008), selenocysteine and selenomethioneine (Amako et al., 2009). Both mushrooms and selenium have been known to show anticarcinogenic activities (Maseko et al., 2014),

The current study was attempted to check whether combination of two nutritive agents will enhance the antiproliferative activity against A549 lung cancer cell lines. In general the crude non - enriched mushroom extracts from four different solvent fractions (hexane, ethanol, methanol and water) exhibited appreciable antiproliferative activity in terms of percent cytotoxicity against cell lines. Anticancer (lung cancer) properties from extracts belonging to various mushrooms are well documented. Intraperitoneal administration of aqueous extracts of *Hypsizigus marmoreus* exhibited inhibitory activity against spontaneous tumour metastasis in

mice with lewis lung carcinoma (Saitoh et al., 1997). Similarly, administration of bioactive extracts of *Phellinus linteus* at a low concentration induced cell - cycle arrest and the high dose responded as apoptosis in human and mouse lung cancer cell (Guo et al., 2007). Blazein extracted from *A. blazei* induced cytotoxicity and morphological change that indicated apoptopic chromatin condensation in LU 99 human lung cancer cell lines (Itoh et al., 2008). Choi et al. (2004) reported cytotoxic effects of *Pleurotus ferulae* extracts on A549, SiHA and HeLa cancer cells lines, wherein ethanolic extracts showed cytotoxicity at an concentration of 10 µg/ml against A549 cell lines and a concentration of 40 µg/ml against SiHA and HeLa cancer cells.

When Se-rich solvent fractions from mushrooms (*A. bisporus*, *V. volvacea* and *P. sajorkaju*) were compared with non-enriched fractions, there was a significant difference in terms of cytotoxicity, with Se-rich mushrooms showing higher activity across all solvent fractions studied. This could be attributed to possible presence of variety of unknown bioactive species in Se hyperaccumulating mushrooms (Bhatia et al., 2013). Apart from the unknown moieties, many known selenium containing compounds such as selenate and selenomethioneine (Combs, 1997; Ip, 1986), selenobetaine and Se-methylselenocysteine (Ip and Ganther, 1990, 1992) are known to inhibit cancer. The presence of such Se-rich moieties might be directly affecting intracellular transduction pathway, triggering specific signaling reactions that may add to cancer inhibition. Zhuo et al. (2004), studied significant decrease in the risk of lung cancer when associated with high selenium exposure in epidemiological evidence from sixteen studies. Our results are supported by Maseko et al. (2014), reporting Se-rich *A. bisporus* as a biologically functional organic Se source, showing activity against oxidative stress and colorectal cancer. Similarly *A.bisporus* enriched with selenium was found to suppress 7,12-dimethylbenz(a)anthracene bioactivation in mammary tissue (Spolar et al., 1999). This study

gives a preliminary idea of effect of selenium on anti-proliferative properties of lung cancer cell lines.

5.7 Identification of selenoergothioneine like moiety

Mushrooms are reported to be potential sources of antioxidant compounds like ergothioneine (ESH) (Lindequist et al., 2005). ESH is known to be synthesized in mycobacterium as well as fungi that include some particular species of mushrooms (Melville et al., 1955), and has known *in-vivo* antioxidant (Akanmu et al., 1991) along with protective role against oxidative damage (Aruoma et al., 1999). Ergothioneine is a naturally available amino acid and is thiourea derivative of histidine, possessing a sulfur atom in the imidazole ring. Selenium has chemical properties similar to sulphur and therefore it is expected that sulphur atom may get replaced with selenium to yield selenium analogue of ergothioneine, a proposed concept of current study. Therefore, an attempt was made to identify selenoergothionine like moiety in Se-rich mushroom extracts. The FTIR and MS spectra gave preliminary indication of presence of such selenium compound like moiety.

The FTIR observations obtained in the study are closely supported by the reports of Al-Mudhaffar and Hasan (2012), where characteristic strong bands due to υ C=Se were observed in the range between 864-987 cm^{-1}. The presence for selenoergothioneine, in Se-rich mushrooms, was also confirmed through MS-ESI data that was in full agreement with the spectra information reported by Yamashita et al. (2010). However, the FTIR and MS observations are only suggestive and need detailed extraction and examination of various fractions.

Further, in mushrooms, cultivated on substrates exogenously supplemented with Se as inorganic selenite, selenoergothioneine was observed to be synthesized along with other organoselenium compounds (Beelman et al., 2007). The selenoergothioneine is formed where

Se effectively replaces S in the ergothioneine moiety. This molecule is expected to exist as two tautomeric forms viz. selenoxo and selenol as suggested by Yamashita et al. (2010). Pioneering work by Beelman et al. (2007), envisaged enhanced potential of selenoergothioneine in mushrooms due to combined benefits of two very strong antioxidant moieties, viz., Se and ergothioneine. Further, reporting the identification of selenoergothioneine (or selenoneine) in tuna fish, Yamashita et al. (2010; 2011) , indicated interaction of these molecules with heme of globins to protect it from auto-oxidation of iron ions under micro-aerophilic conditions.

The present study gives preliminary information regarding presence of selenoergothioneine like moiety and requires furthur extended work. However expression of Se-rich compounds and bioactive moieties as a function of Se uptake is yet to be carried out extensively and therefore furthur characterization of various chemical moieties in Se-rich mushrooms is still required.

In conclusion, the study thus presents the observations on the uptake and bioactivity of selenium by different species of edible mushrooms using selenium hyperaccumulated agricultural residues as substrates. The salient feature of the study is the potential use of waste selenium rich agri-residues of producing selenium fortified mushrooms that can be used as supplements or neutraceuticals for Se deficient population. The observations on the enhanced bioactivity in terms of anti-oxidant and cytotoxic effects, as a function of selenium, further strengths the concept of the potential use of these matrices for neutraceutical applications.

Conclusion

Seven edible mushroom strains namely *Agaricus bisporus* (U-3) and *Volvariella volvacea* (Vv-4) and five strains of *Pleurotus*, viz. *Pleurotus florida* (DMRP-136), *Pleurotus djamor* (DMRP-205), *Pleurotus sajorkaju* (DMRP-112), *Pleurotus citrinopileatus* (DMRP-10) and *Pleurotus ostreatus* (DMRP-2) were cultivated on agricultural residues belonging to selenium rich and non enriched sites. All Se-rich strains were found to accumulate significantly high selenium content than respective non-Se strains, wherein Se-rich *P. djamor* (145.4 µg Se/gDW) was found to accumulate relatively higher total selenium content than other mushroom species and Se-rich *P. citrinopileatus* (26.1 µg Se/gDW) accumulated the lowest.

Total soluble form of selenium in gastrointestinal extracts (GI) of *P. florida* as analysed by ICP-DRC-MS was found to be 105.8 ± 1.6 µg/g dw, which provided an indication that 75% of selenium was appreciably bioavailable to the body. Investigation of the Se molecular fractions revealed that proteins and other high molecular weight selenium-containing molecule were hydrolyzed to peptides and low molecular weight selenocompounds (≤5 kDa based on column calibration). Se speciation in the GI was dominated by selenomethioneine, which represented 73% of the sum of the detected species. Only 2% of the detected species was present as inorganic Se in the form of Se (IV). A number of other low molecular weight selenocompounds generated during the gastrointestinal digestion, accounting for 25% of the chromatographed Se, remained unidentified.

Mushrooms cultivated on naturally Se-enriched substrates did not differ from controls in terms of yield and biological efficiency. Mushroom cultivated on Se-rich agricultural residues were examined for their elemental content with respect to their control. In general, the CHN for

selected mushrooms (*P. florida, P. sajorkaju, P. djamor, P. ostreatus and P. citrinopileatus*) and Fe, Zn, Cu, Mg, Ca content for all seven species (*A. bisporus, V. volvacea, P. florida, P. sajorkaju, P. djamor, P. ostreatus and P. citrinopileatus*) was found to be similar in fruiting bodies of both selenium enriched and control mushrooms.

Se-rich fruiting bodies of all mushrooms have shown significantly higher antioxidant status in terms of all antioxidant assays as compared to control ones. However, when compared at species level, different species have shown varying levels of selenium uptake and therefore varying antioxidant activity. Se-rich *V. volvacea* (17.7 ± 0.5 mg GAE/ g DW) showed highest phenol content, Se-rich *A. bisporus* has shown maximum lipid peroxide inhibition (17.6 ± 4.4 nM MDA/g) and highest free radical scavenging (91.4 ± 1.4 % at 10 mg/ml) with EC_{50} value of 0.056 mg/ml as compared to other Se-enriched species respectively. Similarly, Se-rich *P. sajorkaju* exhibited relatively higher metal chelation with EC_{50} value of 2.12 mg/ml as compared to other Se and NSe species.

To examine the effect of bioactive fractions of selected species (*A. bisporus*, *V. volvacea* and *P. sajorkaju*) on proliferation of A549 lung cancer cells, the crude extracts of mushrooms (Se and NSe) from four different solvents viz. hexane, ethanol, methanol and water were tested. Se-rich bioactive extracts showed significantly higher cytotoxicity than NSe extracts against the cell lines. The Se-rich fraction inhibited the proliferation by 89.6%, 87.9%, 64.9% and 47.5% as compared to 83.2%, 42.3%, 4.4% and 9.4% inhibition by non-Se mushrooms in hexane, ethanol, methanol and water fractions respectively. In case of *V. volvacea*, various solvent fractions showed marginal to significant inhibition of cell proliferation. The Se-rich fraction inhibited the proliferation by 98.87%, 74.8%, 66.0% and 54.0% as compared to 98.71%, 70.5%, 55.0% and 3.0% inhibition by non-Se mushrooms in water, hexane, ethanol and methanol fractions

respectively. Similarly, in case of oyster mushroom (*P. sajorkaju*), ethanol fraction showed maximum inhibition of cell proliferation followed by methanol, water and hexane. The Se-rich fraction inhibited the proliferation by 91.8%, 88.3%, 83.0% and 71.6% as compared to 0%, 11.1%, 55.4% and 51.0% inhibition by non-Se mushrooms in ethanol, methanol, water and hexane fractions respectively. However, when compared at species level among the three different mushroom species; Se-rich bioactive extracts of *P. sajorkaju* showed much potential in inhibiting proliferation of cancer cells in all four fractions.

The Se-rich extracts were analyzed for possible presence of selenium analogue of ergothioneine (selenoergothioneine like moiety) using FTIR and LC-MS. A preliminary indication of presence of selenoergothioneine like moiety, was reached by FTIR wherein the characteristic peak at 931 cm^{-1} indicated the existence of C=Se in the structure. The presence of selenoergothioneine was furthur indicated through results of LC-MS analysis, with a molecular ion $[M+H]^+$ peak at m/z 278.1 corresponding to the molecular formula $C_9H_{15}N_3O_2Se$ and calculated mass of 277.1.

Essentiality of selenium as a micronutrient is gaining ground extensively. A significant component of world's population is at risk of exposure to several diseases due to sub-optimal selenium intake. Therefore the study envisages and proposes the use of Se-hyperaccmulated mushrooms with enhanced bioactivity for preparation of Se supplemented neutrceuticles that can facilitate bioavaliable and accessible Se required to overcome the deficiency. As an added benefit, this approach can also facilitate the re-use of Se-rich agricultural residues which otherwise cause environmental hazard due to burning of post-harvest residues in the region.

References

1. Agrahar-Murugkar D, Subbulakshmi G. (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills, Meghalaya. *Food Chemistry*. 89: 599-603.
2. Akanmu D, Cecchini R, Aruoma OI, Halliwell B. (1991). The antioxidant action of ergothioneine. *Archives of Biochemistry and Biophysics*. 288: 10–16.
3. Akata I, Ergonul B, Kalyoncu F. (2012). Chemical compositions and antioxidant activities of 16 wild edible mushroom species grown in Anatolia. *International Journal of Pharmacology*. 8:134-138.
4. Akil M, Bicer M, Menevse E, Baltaci AK, Mogulkoc R. (2011). Selenium supplementation prevents lipid peroxidation caused by arduous exercise in rat brain tissue. *Braitislavske Lekarske Listy.*112: 314-317.
5. Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW, Lee TS. (2008). Nutritional analysis of cultivated mushrooms in Bangladesh- *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. *Mycobiology*. 36: 228-232.
6. Al-Bayati MA, Raabe OG, Teague SV. (1992). Effect of inhaled dimethyl selenide in the Fischer 344 male rat. *Journal of Toxicology and Environmental Health.* 34: 549–557.
7. Allan CB, Lacourciere GM, Stadtman TC. (1999). Responsiveness of selenoproteins to dietary selenium. *Annual Review of Nutrition*. 19: 1–16.
8. Al-Mudhaffar DMH, Hasan AAA. (2012). Synthesis, characterization and biological activity role of selenium and selenocompounds. *Biomedicine and Pharmacotheraphy*. 57:134-144.
9. Amoako PO, Uden PC, Tyson JF. (2009). Speciation of selenium dietary supplements; formation of S-(methylseleno) cysteine and other selenium compounds. *Analytica Chimica Acta*. 652: 315–323.
10. Andersen A, Lykke SE, Lange M, Bech K. (1982). Trace elements in edible mushrooms. *Publ. 68, Stat. Levnedsmiddelinst., Denmark*, 29 pp (in Danish).
11. Andren AW, Klein DH, Talmi Y. (1975). Selenium in coal-fired steam plant emissions. *Environmental Science and Technology*. 9: 856–858.

12. Angstwurm MWA, Schottdorf J, Schopophil J, Gaertner R. (1999). Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome. *Critical Care Medicine*. 27: 1807-1813.
13. Antonenko K, Kreicbergs V, Duma M, Ozola S. (2013). Selenium effect on rye malt quality. *Proceedings of the Latvian Academy of Sciences, B: Natural. Exact and Applied Sciences*, 67: 394 - 398.
14. Arora SP, Kaur P, Khirawar SS, Chopra RC, Ludri RC. (1975). Selenium levels in fodders and its relationship with Degnala disease. *Indian Journal of Dairy Sciences*. 28: 246-253.
15. Arthur JR. (1994). New metabolic role of selenium. *Proceeding of the Nutrition Society*. 53: 615-624.
16. Aruoma OI, Spencer JPE, Mahmood N. (1999). Protection against oxidative damage and cell death by the natural antioxidant ergothioneine. *Food and Chemical Toxicology*. 37: 1043–1053.
17. Ashour MN, Salem SI, El-Gadban HM, Elwa NM, Basu TK. (1999). Antioxidant status in children with protein energy malnutrition (PEM) living in Cairo, Egypt. *European Journal of Clinical Nutrition.* 53: 669-673.
18. Baeten JM, Mostad SB, Hughes MP, Overbaugh J, Bankson DD, Mandaliya K, Ndinya-Achola JO, Bwayo JJ, Kreiss JK. (2001). Selenium deficiency is associated with shedding of HIV-1-infected cells in the female genital tract. *Journal of Acquired Immune Deficiency Syndrome.* 26: 360–364.
19. Bajaj M, Eiche E, Neumann T, Winter J, Gallert G. (2011). Hazardous concentration of Se in soil and ground water in North West India. *Journal of Hazardous Materials*. 189: 640-646.
20. Banuelos GS, Lin ZQ, Wu L, Terry N. (2002). Phytoremediation of selenium-contaminated soils and water: fundamentals and future prospects. *Reviews on Environmental Health.* 17: 291-306.
21. Barclay MNI, MacPherson A, Dixon J. (1995). Selenium content of a range of UK foods. *Journal of Food Composition and Analysis.*8: 307-318.
22. Barlow SM. (1990). Toxicological aspects of antioxidants used as food additives. In: Hudson, BJH (ed.), *Food Antioxidants*, London: Elsevier, pp. 253– 307.

23. Barrett CW, Singh K, Motley AK, Lintel MK, Matafonova E, Bradley AM, Ning W, Poindexter SV, Parang B, Reddy VK, Chaturvedi R, Fingleton BM, Washington MK, Wilson KT, Davies SS, Hill KE, Burk RF, Williams CS. (2013). Dietary selenium deficiency exacerbates DSS-induced epithelial injury and AOM/DSS-induced tumorigenesis. PLoS One 8: e67845.

24. Barros L, Bapista P, Correia DM, Morais JS, Ferreira ICFR. (2007). Effects of conservation treatment and cooking on chemical composition and antioxidant activity of Portuguese wild edible mushroom. *Journal of Agricultural and Food Chemistry*. 55: 4781-4788.

25. Barros L, Ventuizini BA, Baptista P, Estevinho LM, Ferreira I. (2008). Chemical composition and biological properties of Portuguese wild mushrooms: A comprehensive study. *Journal of Agricultural and Food Chemistry*. 56: 3856-3862.

26. Bartosz G. (1995). *Druga Twarz Tlenu (The Second Face of Oxygen)*. Wydawnictwo Naukowe PWN, Warzszawa (in Polish).

27. Battin EE, Perron NR, Brumaghi JL. (2006).The central role of metal coordination in selenium antioxidant activity. *Inorganic Chemistry.* 45: 499-501.

28. Baum MK, Shor-Posner G. (1998). Micronutrient status in relationship to mortality in HIV-1 disease. *Nutrition Reviews.* 56: S135-S139.

29. Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB. (1997). High risk of HIV- related mortality is associated with selenium deficiency. *Journal of Acquired Immune Deficiency Syndrome Human Reterovirology*.15: 370-400.

30. Beckett GJ, Arthur JR. (2005). Selenium and endocrine systems. *Journal of Endocrinology.* 184: 455–465.

31. Beckett GJ, Beddows SE, Morrice PC. (1987). Inhibition of hepatic de-iodination of thyroxine is caused by selenium deficiency in rats. *Biochemical Journal.* 248: 443–447.

32. Beelman RB, Dubost NJ, Peterson DG, Hausman M. (2007). *Phytonutrient Compositions from Mushrooms or Filamentous Fungi and Methods of Use*. US Patent # US 2007/0244175 A1.

33. Bhatia P, Aureli F, Amato M, Prakash R, Cameotra SS, Tejo Prakash N, Cubadda F. (2013): Selenium bioaccessibility and speciation in biofortified *Pleurotus* mushrooms grown on selenium-rich agricultural residues. *Food Chemistry.* 140: 225-230.

34. Birringer, M, Pilawa S, Flohe, L. (2002). Trends in selenium biochemistry, *Nature Product Reports*. 19: 693-718.

35. Bleys J, Navas-Acien A, Guallaor E. (2007). Serum selenium and diabetes in U.S. *Adults Diabetes Care*. 30: 829–834.

36. Bosschaerts T, Guilliams M, Noel W, Hérin M, Burk RF, Hill KE, Brys L, Raes G, Ghassabeh GH, De Baetselier P, Beschin A. (2008). Alternatively activated myeloid cells limit pathogenicity associated with African Trypanosomiasis through the IL-10 inducible gene selenoprotein P. *Journal of Immunology*. 180: 6168–6175.

37. Boyne R, Arthur JR, (1986). The response of selenium deficient mice to *Candida albicans* infection. *The Journal of Nutrition*. 116: 816-822.

38. Brown KM, Arthur, JR. (2001). Selenium, selenoproteins and human health: a review. *Public Health Nutrition*. 4: 593-599.

39. Brown TA, Shrift A. (1982). Selenium: toxicity and tolerance in higher plants. *Biological Reviews*. 57: 59–84.

40. Bueno M, Pamier F, Potin-Gautier M. (2007). Determination of organic and inorganic selenium species using HPLC-ICP-MS (Reference information- HPLC-ICP-MS, Agilent technologies, USA).

41. Bugel S, Sandstrom B, Larsen EH, Skibsted LH. (2002). Is selenium from animal sources bioavailable? In: *Proceedings of 11th Symposium on Trace Elements in Man and Animals*, Berkeley, USA.

42. Burk RF, Hill KE. (1993). Regulation of selenoproteins. *Annual Review of Nutrition*. 13: 65-81.

43. Burk RF, Hill KE, Motley AK. (2001). Plasma selenium in specific and non-specific forms. *Biofactors*. 14: 107-114.

44. Burk RF, Hill KE, Motley AK. (2003). Selenoprotein metabolism and function: Evidence for more than one function for SeP. *Journal of Nutrition*. 133: 1517S-1520S.

45. Burk RF, Levander OA. (1999). Selenium. In: Shils ME, Olson JA, Shike M, Ross AC (eds.), *Modern Nutrition in Health and Disease*, Baltimore: Williams & Wilkins, pp 265–276.

46. Buswell JA, Cai YI, Chang, ST. (1993). Fungal and substrate-associated factors affecting the ability of individual mushroom species to utilize different lignocellulosic growth

substrates, In: Chang ST, Buswell JA, Chiu S (eds.) *Mushroom Biology and Mushroom Products*. Hong Kong: The Chinese University Press, pp. l41-149.

47. Byrne AR, Kosta L, Ravnik V. (1976). Trace element concentrations in higher fungi. *Science of the Total Environment.* 6: 65–78.
48. Campa A, Shor-Posner G, Indacochea F. (1999). Mortality risk in selenium-deficient HIV-positive children. *Journal of Acquired Immune Deficiency Syndrome*. 20: 508–513.
49. Cankur O, Yathavakilla SKV, Caruso JA. (2006). Selenium speciation in dill (*Anethum graveolnes L.*) by ion pairing reversed phase and cation exchange HPLC with ICP-MS detection. *Talanta*. 70: 784–790.
50. Cartes P, Gianfreda L, Mora LM. (2005). Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite form. *Plant and Soil.* 256: 359–367.
51. Carvalho KM, Gallardo-Williams MT, Benson RF, Martin DF. (2003). Effects of selenium supplementation on four agricultural crops. *Journal of Agricultural and Food Chemistry*. 57: 704–709.
52. Chang ST, Lau OW, Cho KY. (1981).The cultivation and nutritional value of *Pleurotus sajor-caju*. *European Journal of Applied Microbiology and Biotechnology*. 12: 58-62.
53. Chansler MW, Mutanen M, Morris VC, Levander OA. (1986). Nutritional bioavailability to rats of selenium in Brazil nuts and mushrooms. *Nutrition Research*. 6:1419–1428.
54. Charter RA, Tabatabai MA, Schafer JW. (1995). Arsenic, molybdenum, selenium, and tungsten contents of fertilizers and phosphate rocks. *Communication in Soil Science and Plant Analysis*. 26: 3051–3062
55. Cheung LM, Cheung PCK, Ooi VEC. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*. 81: 249-255.
56. Cheung LM, Cheung PCK. (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chemistry*. 89: 403-409.
57. Chi-Chen YC, Sosnoski DM, Gandhi UH, Novinger LJ, Prabhu KS, Mastro AM. (2009). Selenium modifies the osteoblast inflammatory stress response to bone metastatic breast cancer. *Carcinogenesis*. 30: 1941-1948.
58. Choi D, Cha WS, Kang SH, Lee BR. (2004). Effect of *Pleurotus ferulae* extracts on viability of human lung cancer and cervical cancer cell lines. *Biotechnology and Bioprocess Engineering*. 9: 356-361.

59. Christophersen OA, Lyons G, Haug A, Steinnes E. (1995). Selenium. In: Alloway, BJ (ed.), *Heavy Metals in Soils*. Glasgow: Blackwell, pp. 429-463.

60. Chu YH, Chang CL, Hsu HF. (2000). Flavanoid content of several vegetables and their antioxidant activity. *Journal of Science of Food and Agriculture.* 80: 561-566.

61. Clark LC, Combs GF, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL, Park HK, Sanders BB, Smith CL, Taylor JR. (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *Journal of the American Medical Association*. 276: 1957–1963.

62. Clark LC, Graham GF, Crounse RG, Grimson R, Hulka B, Shy CM. (1984). Plasma selenium and skin neoplasms: a case control study. *Nutrition and Cancer*. 6: 13–21.

63. Clark LC, Hixson LJ, Combs GF, Jr, Reid ME, Turnbull BW, Sampliner RE. (1993). Plasma selenium concentration predicts the prevalence of colorectal adenomatous polyps. *Cancer Epidemiology, Biomarkers and Prevention.* 2: 41–46.

64. Combs GF. (1990) Growing interest in selenium. *Western Journal of Medicine.* 153: 192-194.

65. Combs GF, Gray WP. (1998). Chemopreventive agents: selenium. *Pharmacology and Therapeutics.* 79: 179-192.

66. Combs GF (2001). Selenium in global food systems. *British Journal of Nutrition*. 85: 517–547.

67. Combs GF. (1988). Selenium in foods. In: Chichester CO, Schweiger BS. (eds.), *Advances in Food Research*, San Diego: Academic Press, pp. 85–113.

68. Combs GF. (1997). Selenium and Cancer. In: Garewal HS, (ed.), *Antioxidants and Disease Prevention*, New York: CRC Press, pp. 97–113.

69. Contempre B, Le Moine O, Dumont JE, Denef JF, Many MC.(1996). Selenium deficiency and thyroid fibrosis. A key role for macrophages and TGF-α. *Methods in Enzymology.* 124: 7-15.

70. Costa-Silva F, Marques G, Matos CC, Barros IRNA, Nunes FM. (2011). Selenium contents of Portuguese commercial and wild edible mushrooms. *Food Chemistry*, 26: 91-96.

71. Cremades O, Herrero MMD, Aguilar PC, Gil JFG, Fontiveros E, Morgado BR, Parrado J, Bautista J. (2012). Preparation and characterization of selenium enriched mushroom aqueous enzymatic extract (MAEE) obtained from the white button mushroom (*Agaricus bisporus*). *Food Chemistry*. 133: 1538-1543.

72. Cubadda F, Aureli F, Ciardullo S, D'Amato M, Raggi A, Aachary R, Tejo Prakash N. (2009). Total selenium and selenium speciation in food crops from a seleniferous area of Punjab, In: Proceedings of 3rd IUPAC Symposium on Trace Elements in Food, Italy. pp.153.

73. Cubadda F, Aureli F, Ciardullo S, D'Amato M, Raggi A, Acharya R, Tejo Prakash N. (2010). Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *Journal of Agricultural and Food Chemistry*. 58: 2295–2301.

74. Da Silva MC, Naozuka J, Oliveira PV, Vanetti MC, Bazzolli DM, Costa NM, Kasuya MC. (2010). *In vivo* bioavailability of selenium in enriched *Pleurotus ostreatus* mushrooms. *Metallomics*. 2:162-166.

75. da Silva MC, Naozuka J, da Luz JMR, de Assuncao LS, Oliveira PV, Vanetti MCD, Bazzolli DMS, Kasuya MCM. (2012). Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chemistry.* 131: 558-563.

76. Davis ZF, Lu Y, Thompson JT, Prabhu KS, Reddy PV, Sordillo LM, Reddy CC. (2002). Nuclear factor- kappa B mediates over expression of cyclooxygenase-2 during activation of RAW 246.7 macrophages in selenium deficiency. *Free Radical Biology and Medicine.* 32: 890-897.

77. Dernovics M, Stefanka Z, Fodor P. (2002). Improving selenium extraction by sequential enzymatic processes for Se-speciation of selenium-enriched *Agaricus bisporus*. *Analytical and Bioanalytical Chemistry*. 372: 473–480.

78. Desai D, Kaushal N, Gandhi UH, Arner RJ, D'Souza C, Chen G, Vunta H, El-Bayoumy K, Amin S, Prabhu KS (2010). Synthesis and evaluation of the anti-inflammatory properties of selenium-derivatives of celecoxib. *Chemico-Biological Interactions*. 88: 446-456.

79. Dey S, Stafford R, Deb Ro, MK, Bhattacharjee CR, Khathing DT, Bhattacharjee PC, Dkhar, P. S. (1999). Metal toxicity and trace element deficiency in some wild animals

species from North-East India, as revealed by cellular, bio-inorganic and behavioural studies. *Current Science*. 77: 276–280.

80. Dhillon KS, Dhillon SK. (1991). Selenium toxicity in soils, plants and animals in some parts of Punjab, India. *International Journal of Environmental Studies.* 37: 15–24.
81. Dhillon KS, Dhillon SK. (1997). Distribution of seleniferous soils in north-west India and associated toxicity problems in soil-plant-human continuum. *Land Contamination and Reclamation*. 5:313-322.
82. Dhillon KS, Dhillon SK. (2001). Restoration of selenium-contaminated soils. In: Iskandar IK. (ed.), *Environmental Restoration of Metals-contaminates Soils*. Boca Raton: CRC Press, pp. 199–227.
83. Dhillon KS, Dhillon SK. (2003a). Distribution and management of seleniferous soils. In: *Advances in Agronomy*, 79: 119–184.
84. Dhillon KS, Dhillon SK. (2003b). Quality of underground water and its contribution towards selenium enrichment of the soil–plant system for a seleniferous region of northwest India. *Journal of Hydrology.* 272:120-130.
85. Dhillon KS, Bawa SS, Dhillon SK. (1992). Selenium toxicity in some plants and soils of Punjab. *Journal of Indian Society of Soil Science*. 40: 132–136.
86. Diaz Huerta V, Fernandez Sanchez ML, Sanz-Medel A. (2005). Qualitative and quantitative speciation analysis of water soluble selenium in three edible wild mushrooms species by liquid chromatography using post-column isotope dilution ICP–MS. *Analytica Chimica Acta*. 538: 99–105.
87. Dinis TCP, Madeira VMC, Almeida LM. (1994). Action of phenolic derivatives (acetoaminophen,salicylate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics.* 315:161-169.
88. Diwadkar-Navsariwala V, Diamond AM. (2004). The link between selenium and chemoprevention: A case for selenoproteins. *Journal of Nutrition*. 134: 2899-902.
89. Dodig S, Cepelak I. (2004). The facts and controversies about selenium. *Acta Pharmacologica.* 54: 261-276.

90. Dong Y, Lisk D, Block E, Ip C. (2001) Characterization of the biological activity of g-glutamyl-Se-methylselenocysteine: A novel, naturally occurring anticancer agent from garlic. *Cancer Research.* 61:2923–2928.

91. Doran JW, Alexander M. (1977). Microbial formation of volatile Se compounds in soil. *Soil Science Society of America Journal*. 40: 687–690.

92. Dowdle PR, Oremland RS (1998). Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. *Environmental Science and Technology.* 32: 3749–3755.

93. Draize JH, Beath OA. (1935). Observation on pathology of blood staggers and alkali disease. *Journal of American Veterinary Medical Association*. 39: 753-763.

94. Dumont E, Vanhaeke F, Cornells R. (2006). Selenium speciation from food sources to metabolites a critical review. *Analytical and Bioanalytical Chemistry*. 385: 1304-1323.

95. Dundlar A, Acay H, Yildiz A. (2008). Yield performances and nutritional contents of three oyster mushrooms species cultivated on wheat stalk. *African Journal of Biotechnology*. 7: 3497- 3501.

96. El-Bayoumy K. (1991). The role of selenium in cancer prevention. In: deVita, VT, Hellman S, Rosenberg SS (eds.), *Practice of Oncology* (4th Ed). Philadelphia: Lippincott. pp. 1–15.

97. El-Demerdash FM. (2004). Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *Journal of Trace Elements in Medicine and Biology*. 18: 113–121.

98. Elteren JT, Woroniecka UD, Kroon KJ. (1998). Accumulation and distribution of selenium and cesium in the cultivated mushroom *Agaricus bisporus* - A radiotracer-aided study. *Chemosphere*. 36:1787–1798.

99. Ermakov VV. (1992). Biogeochemical regaining problems and the biochemical selenium provinces of the former USSR. *Biological Trace Element Research*. 33: 171-185.

100. Estrada AER, Lee HJ, Beelman RB, Jimenez-Gasco MD, Royse DJ. (2009). Enhancement of the antioxidants ergothioneine and selenium in *Pleurotus eryngii var. eryngii* basidiomata through cultural practices. *World Journal of Microbiology and Biotechnology*, 25: 1597-1607.

101. Falandysz J. (2008). Selenium in edible mushrooms. *Journal of Environmental Science and Health Part C-Environmental Carcinogenesis and Ecotoxicology Reviews*. 26:256–299.

102. FAO/WHO (2002). Human vitamin and mineral requirements. Report of a joint FAO/WHO consultation, Bangkok, Thailand. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

103. Fatma M, Demerdash E. (2004). Antioxidant effect of Vitamin E and selenium on lipidperoxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *Journal of Trace Element and Medical Biology.* 18:113-121.

104. Fellowes JW, Pattricka RAD, Boothman C, AlLawati WMM, VanDongen BE, Charnock JM, Lloyd JR, Pearce CI. (2013). Microbial selenium transformations in seleniferous soils. *European Journal of Soil Sci*ences. 64: 629–638

105. Ferrarello CN, Campa RF, Carraso JF, Sanz-Medel A. (2002). Speciation of metallothionein-like proteins of the mussel *Mytilus edulis* by orthogonal separation mechanisms with inductively coupled plasma-mass spectrometry detection: effect of selenium administration. *Spectrochimica Acta Part B: Atomic spectroscopy*. 57: 439-449.

106. Ferreira ICFR, Baptista AS, Vilas-Boas M, Barros L. (2007). Free radical scavenging capacity and reducing power of wild edible mushrooms from Northeast Portugal: Individual cap and stipe study. *Food chemistry*. 100: 1511-1516.

107. Finley J, Matthys L, Shuler T and Korynta E. (1996). Selenium content of foods purchased in North Dakota. *Nutrition Research.* 16: 723–728.

108. Finley JW, Davis C, Feng Y. (2000). Selenium from high-selenium broccoli protects rats from colon cancer. *Journal of Nutrition*, 130: 2384-2389.

109. Finley JW, Ip C, Lisk DJ, Davis CD, Hintze KJ, Whanger PD. (2001). Investigation on the cancer-protective properties of high-Se broccoli. *Journal of Agricultural Food Chemistry.* 49:2679–2683.

110. Finley JW. (2006). Bioavaliability of selenium from foods. *Nutrition Reviews*. 64:146-151.

111. Fleming GA. (1962). Selenium in Irish soils and plants. *Soil Sciences*. 94: 28–35.

112. Fleming GA. (1980). Essential micronutrients II: Iodine and selenium. In: Davis BE (ed.), *Applied Soils Trace Elements* (1st Ed.), New York: John Wiley & Sons, pp 199–234.

113. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. (2006). Selenium and coronary heart disease:A meta-analysis. *American Journal of Clinical Nutrition*./84:/762-73.

114. Flynn A, Hirnoven T, Mensisk GB, Ocke MC, Serra-Majem L, Stos K, Szponar L, Tetenus I, Turrini A, Fletcher R, Wildemann T. (2009). Intake of selected nutrients from foods, from fortification and from supplements in various European countries. *Journal of Food and Nutrition Research* (Suppl.) 53: 10.

115. Fordyce F. (2005). Selenium deficiency and toxicity in the environment. In: Selinus O. (ed.). *Essentials of Medical Geology* (1st Ed.), Uppsala: Elsevier, pp. 373–415.

116. Franke and Painter E. (1938). A study on toxicity and selenium content of seleniferous diets: with statistical consideration. *Cereal Chemistry*. 15: 1-24.

117. Gandhi H, Ujjwal R, Nagaraja T, Prabhu KS. (2013). Selenoproteins and their role in oxidative stress and inflammation. *Current Chemical Biology*. 7: 65-73.

118. Gandhi UH, Kaushal N, Ravindra KC, Hegde S, Nelson SM, Narayan V, Vunta H, Paulson RF, Prabhu KS. (2011). Selenoprotein-dependent upregulation of hematopoietic prostaglandin D_2 synthase in macrophages is mediated through the activation of peroxisome proliferator-activated receptor (PPAR)γ. *Journal of Biological Chemistry,* 286: 27471-27482.

119. Ganther HE. (1999). Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. *Carcinogenesis,* 20:1657-1666.

120. Ganther HE. (2001). Selenium metabolism and mechanism of cancer prevention. *Advances in Experimental Medicine and Biology*. 492: 119-130.

121. Gartner R, Angstwurm M. (1999). Significance of selenium in intensive care medicine. Clinical studies of patients with SIRS/sepsis syndrome. *Medizinische Klinik,* 94: 54-57.

122. Gartner R, Gasnier BC. (2003). Selenium in the treatment of autoimmune thyroiditis. *Biofactors*. 19: 165-170.

123. Gast CH, Jansen E, Bierling J, Haanstra L. (1988). Heavy metals in mushrooms and their relationship with soil characteristics. *Chemosphere*. 17: 789-799.

124. Gazdik F, Kadrabova J, Gazdikova K.(2002). Decreased consumption of corticosteroids after selenium supplementation in corticoid-dependent asthmatics. *Bratislavske Lekarske Listy*.103:22–25.

125. Geregley V, Kubachka KM, Mounicou S, Fodor P, Caruso JA. (2006). Selenium speciation in *Agaricus bisporus* and *Lentinula edodes* mushroom proteins using multi-dimensional chromatography coupled to inductively coupled mass spectrometry. *Journal of Chromatography A*, 1101: 94-102.

126. Ghadirian P, Maisonneuve P, Perret C, Kennedy G, Boyle P, Krewski D, Lacroix AA. (2000). Case-control study of toenail selenium and cancer of the breast, colon, and prostate. *Cancer Detection and Prevention*, 24: 305-13.

127. Goh KH, Lim TT. (2004). Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. *Chemosphere*. 55: 849-859.

128. Gordon MH. (1990). The mechanism of antioxidant action *in vitro*. In: Hudson BJF. (ed.), *Food Antioxidants*: New York: Elsevier, pp. 1-18.

129. Griffith HW, (ed.). (1995). Complete Guide to Vitamins Minerals and Supplements. New York: Fisher,:100–126.

130. Guerinot ML, Salt DE (2001) Fortified foods and phytoremediation: Two sides of the same coin. *Plant Physiology*. 125: 164–167

131. Guo JJ, Zhu TB, Collins L, Xiao ZXJ, Kim SH, Chen CY. (2007). Modulation of lung cancer growth arrest and apoptosis by *Phellinus linteus*. *Molecular Carcinogenesis*. 46:144–154.

132. Gupta RC, Kwatra MS, Singh N. (1982). Chronic selenium toxicity as a cause of hoof and horn deformities in buffalo, cattle and goat. *Indian Veterinary Journal*. 59:738.

133. Halliwell B, Murcia HA, Chirco S, Aruoma OI. (1995). Free radicals and antioxidants in food and *in vivo*: What they do and how they work. *Critical Reviews in Food Science and Nutrition.* 35: 7–20.

134. Hamilton, S.J. (2004). Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment*. 326:1-31.

135. Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, Ferrerira, ICFR. (2011). Targeted metabolites analysis in wild *Boletus* species. *LWT-Food Science and Technology*. 44: 1343-1348.

136. Higaki JN, Fletterick RJ, Craik CS. (1992). Engineered metalloid regulation in enzymes. *Trends in Biochemical Sciences*.17:100–104.

137. Hinojosa L, Ruiz J, Marchante JM, Gacía JI, Sanz-Medel A, (2006). Selenium bioaccessibility assessment in selenized yeast after *in vivo* gastrointestinal digestion using two-dimensional chromatography and mass spectrometry. *Journal of Chromatography A.* 110:108-116.

138. Hira CK, Partal K, Dhillon, KS. (2003). Dietary selenium intake by man and woman in high and low selenium areas of Punjab. *Punjab Health Nutrition*. 7: 39-43.

139. Holben DH, Smith AM. (1999). The diverse role of selenium within selenoproteins: A review. *Journal of American Dietetic Association.* 99:836–843.

140. Hong J, Bañuelos GS, Fowler T, Lin Z-Q. (2011). Use of selenium-laden plant materials from phytoremediation to produce selenium-biofortified edible mushrooms.In: *Proceedings of 11th International Conference on the Biogeochemistry of Trace Elements*, Florence, Italy, pp. 349– 350.

141. Hu Y, McIntosh GH, Le Leu RK, Woodman R,Young GP. (2008). Suppression of colorectal oncogenesis by selenium-enriched milk proteins: Apoptosis and Kras mutations. *Cancer Research*. 68: 4936–4944.

142. Hu Y, McIntosh GH, Le Leu RK,Young GP. (2010). Selenium-enriched milk proteins and selenium yeast affect selenoprotein activity and expression differently in mouse colon. *British Journal of Nutrition*, 104: 17–23.

143. Huang LC. (2000). *Antioxidant properties and polysaccharide composition analysis of Antrodia camphorate and Agaricus blazei*. MS Thesis, Chung-Hsing University, Taichung, Taiwan.

144. Huang Z, Rose AH, Hoffmann PR. (2012). The role of selenium in inflammation and immunity: From molecular mechanisms to therapeutic opportunities. *Antioxidants and Redox Signaling.*16:705–743.

145. Hung PV, Nhi NNY. (2012). Nutritional composition and antioxidant capacity of several edible mushrooms grown in the southern Vietnam. *International Food Research Journal*, 19: 611-615.

146. Hurst R, Collings R, Harvey LJ, King M, Hooper L, Bouwman J, Gurinovic M, Fairweather SJ. (2014). EURRECA-Estimating selenium requirements for deriving dietary reference values, *Critical Reviews in Food Science and Nutrition*. DOI:10.10801/10408398.2012.742861.

147. Ihnat M. (1989). Occurence and distribution of Selenium in children with protein-calorie malnutrition (PEM) living in Cairo, Egypt. *European Journal of Clinical Nutrition*. 52: 669-673.

148. Imran MM, Raja MM, Basith JA, Asarudeen A. (2011). Determination of total phenol, flavanoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *International Food Research Journal.* 18: 574-577.

149. Institute of Medicine (IOM). (2000). Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington DC: National Academy Press, pp. 506.

150. Ip C, Birringer M, Block E, Kotrebai M, Tyson JF, Uden PC, Lisk DJ. (2000). Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *Journal of Agricultural and Food Chemistry*. 48: 2062–2070.

151. Ip C, Ganther H. (1990). Activity of methylated forms of selenium in cancer prevention. *Cancer Research*. 50, 1206–1211.

152. Ip C, Ganther H. (1992). Comparison of Se and S analogs in cancer prevention .*Carcinogenesis* 13:1167-1170

153. Ip C, Hayes C. (1989). Tissue selenium levels in selenium-supplemented rats and their relevance in mammary cancer protection. *Carcinogenesis.* 10: 921–925.

154. Ip C, Lisk D. (1994). Characterisation of tissue selenium profiles and anticarcinogenic responses in rats fed natural sources of selenium-rich products. *Carcinogenesis.* 15: 573–576.

155. Ip C, Hayes C, Budnick R, Ganther H. (1991). Chemical form of selenium, critical metabolites, and cancer prevention. *Cancer Research.* 51:595–600.

156. Ip C. (1986). The chemopreventive role of selenium in carcinogenesis. *Journal of the American College of Toxicology*. 5: 7–20.

157. Ip C. (1998). Lessons from basic research in selenium and cancer prevention. *Journal of Nutrition*, 128: 1845-1854.

158. Itoh H, Ito H, Hibasami H. (2008). Blazein of a new steroid isolated from *Agaricus blazei Murrill* (himematsutake) induces cell death and morphological change indicative of apoptotic chromatin condensation in human lung cancer LU99 and stomach cancer KATO III cells. *Oncology Reports*. 20: 1359–1361.

159. Jacobs LW. (ed.) (1989). *Selenium in Agriculture and the Environment*. SSSA Publication 23, Madison: SSSA, pp. 233.

160. Jahnova E, Horvathova M, Gazdik F, and Weissova S. (2002). Effects of selenium supplementation on expression of adhesion molecules in corticoid-dependent asthmatics. *Bratislavske Lekarske Listy.* 103: 12–16.

161. Jaiswal SK, Prakash R, Acharya R, Reddy AVR, Tejo Prakash N. (2012a). Selenium content in seed, oil and oil cake of Se hyperaccumulated *Brassica juncea* (Indian mustard) cultivated in a seleniferous region of India. *Food Chemistry*. 134: 401-404.

162. Jaiswal SK, Prakash R, Acharya R, Nathaniel TN, Reddy AVR, Tejo Prakash N. (2012b). Bioaccessibility of selenium from Se-rich food grains of the seleniferous region of Punjab, India as analyzed by instrumental neutron activation analysis. *CYTA-Journal of Food.* 10: 160-164.

163. Jaworska G, Bernas E, Mickowska B. (2011). Effect of production process on the amino acid content of frozen and canned *Pleurotus ostreatus* mushroom. *Food Chemistry*. 125: 936-943.

164. Judson GJ, Reuter DJ. (1998). *Soil Analysis: An Interpretation Manual*, Urrbrae, South Australia Research and Development Institute (SARDI), pp. 329.

165. Kalac P. (2009). Chemical composition and nutritional value of Eurpoean species of wild growing mushrooms: A review. *Food Chemistry*. 113: 9-16.

166. Kalac P. (2010). Trace elemental contents in European species of wild growing edible mushrooms. A review for the period 2000-2009. *Food Chemistry*. 122: 2-15.

167. Kalac P, Svoboda L. (2000). A review of trace element concentrations in edible mushrooms. *Food Chemistry.* 69: 273-281.

168. Kauf E, Dawczynski H, Jahreis G, Janitsky E, Winnefeld K. (1994). Sodium selenite therapy and thyroid-hormone status in cystic fibrosis and congenital hypothyroidism. *Biological Trace Element Research*. 40: 247-253.

169. Keshan Disease Research Group (1979a). Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. *Chinese Medical Journal*. 92: 477-482.

170. Keshan Disease Research Group (1979b). Observations on the effects of sodium selenite on the prevention of Keshan Disease. *Chinese Medical Journal*. 92: 471-476.

171. Khanna, PK. (2003). Mushroom Growing, Singh AP, (eds.) *Mushroom Cultivation*. Ludhiana: Punjab Agricultural University Press, pp. 1-60.

172. Kim MY, Seguin P, Ahn JK, Kim JJ, Chun SC, Kim EH, Seo SH, Kang EY, Kim SL, Park YJ, Ro HM, Chung IM. (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *Journal of Agricultural and Food Chemistry*. 56: 7265-7270.

173. Kiremidjian-Schumacher L, Roy M, Wishe HI, Cohen MW, Stotzky G. (1994). Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells. *Biological and Trace Element* Research. 41: 115-127.

174. Kohrle J. (1999). The trace element selenium and the thyroid gland. *Biochimie.* 81: 527-533.

175. Kosta L, Byrne AR, Zelenko V, Stegnar P, Dermelj M, Ravnik V. (1974). Studies on the uptake, distribution and transformations of mercury in living organisms in the Idrija region and comparative areas. *Vestnik Slovenskega Kemijskega Drustva.* 21: 49-76.

176. Kowluru RA, Tang J, Kern TS. (2001). Abnormalities of retinal metabolism in diabetes and experimental galactosemia.VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes*. 50:1938–1942.

177. Kozarski M, Klaus A, Niksic M, Jakovljevic D, Helsper JPFG, Van Griensven LJLD. (2011). Antioxidative and immunomodulating activities of polysaccharides extracts of the medicinal mushrooms *Agaricus bisporus*, *Agaricus brasiliensis*, *Ganoderma lucidium* and *Phellinus linteus*. *Food Chemistry*. 129: 1667-1675.

178. Krauskopf KB. (1982). *Introduction to Geochemistry* (2nd Ed.), Singapore: McGraw-Hill, pp. 617.

179. Krehl S, Loewinger M, Florian S, Kipp AP, Banning A, Wessjohann LA, Brauer MN, Iori R, Esworthy RS, Chu FF, Brigelius-Flohé R. (2012). Glutathione peroxidase-2 and selenium decreased inflammation and tumors in a mouse model of inflammation-associated carcinogenesis whereas sulforaphane effects differed with selenium supply. *Carcinogenesis*. 33: 620–628.

180. Kurihara M, Kumagai K, Nakae Y, Nishino I, Nonaka I. (2000). Two sibling patients with non-Fukuyama type congenital muscular dystrophy with low serum selenium levels – therapeutic effects of oral selenium supplementation. *No To Hattatsu.* 32: 346-351.

181. Kurz B, Jost B, Schunke M. (2002). Dietary vitamins and selenium diminish the development of mechanically induced osteoarthritis and increase the expression of antioxidative enzymes in the knee joint of STR/1N mice. *Osteoarthritis and Cartilage*. 10: 119–126.

182. Laborde J, Lanzi G, Francescutti B, Giordani E. (1993). Indoor Composting: General principles and large scale development in Italy. In: Chang ST, Buswell JA, Chiu SW (eds.), *Mushroom Biology and Mushroom Products*. Hong Kong: The Chinese University Press, pp. 93-113.

183. Laclaustra M, Navas-Acien A, Stranges S, Ordovas J M, Guallar E. (2009). Serum selenium concentrations and diabetes in U.S. Adults: National health and nutrition examination survey (NHANES) 2003–2004. *Environmental Health Perspectives* 117:1409-1413.

184. Lakin HW. (1972). Selenium accumulation in soils and its absorption by plants and animals. *Geological Society of America Bulletin*. 83: 181-189.

185. Latiff LA, Daran ABM, Mohammed AB. (1996). Relative distribution of minerals in the pileus and stalk of some selected edible mushroom. *Food Chemistry.* 56: 115-121.

186. Lavola A, Aphalo PJ, Lehto T. (2011) Boron and other elements in sporophores of ectomycorrhizal and saprotrophic fungi. *Mycorrhiza.* 21: 155-165

187. Lee YL, Jian AY, Mau JL. (2009). Composition and non-volatile taste components of *Hypsizigus marmoreus*. *LWT-Food Science and Technology*. 42: 594-598.

188. Lemly AD. (1997). Environmental implications of excessive selenium. *Biomedical and Environmental Sciences.* 10: 415-435

189. Lepsova M, Mejstrik V. (1988). Accumulation of trace elements in the fruiting bodies of macrofungi in the Krušné hory mountains, Czechoslovakia. *Science of the Total Environment.* 76: 117–128

190. Letavayova L, Vlckova V, Brozmanova J. (2006). From chemoprevention to DNA damage. *Toxicol*ogy. 227: 1-14.

191. Levander OA. (1972). Metabolic interrelationships and adaptations in selenium toxicity. *Annals New York Academy of Sciences*. 192: 181-192.

192. Levander OA. (1987). A global view of human selenium nutrition. *Annual Review of Nutrition.* 7: 227-50.

193. Levander OA, Beck MA. (1997). Interacting nutritional and infectious etiologies of Keshan disease. Insights from Cox-sackie virus B-induced myocarditis in mice deficient in selenium or Vitamin E. *Biological Trace Element Research*. 56: 5-21.

194. Levander OA, Beck MA. (1999). Selenium and viral virulence. *British Medical Bulletin*. 55: 528–533.

195. Levesque M, Vendette ED. (1970). Selenium determination in soil and plants. *Canadian Journal of Soil Science*. 51: 85-93.

196. Li GS, Wang F, Kang DR, Li C. (1985). Keshan disease: An endemic cardiomyopathy in China. *Human Pathol*ogy. 16: 602-609.

197. Lin HC. (1999). *Evaluation of Taste Quality and Antioxidant Properties of Edible and Medicinal Mushrooms*. MS Thesis. National Chung Hsing University. Taichung. Taiwan.

198. Lin ZQ, Haddad S, Hong J, Morrissy J. (2014). Use of selenium-contaminated plants from phytoremediation for production of selenium-enriched edible mushrooms. In: Banuelos G, Lin Z-Q, Yin X (eds.), Selenium in the Environment and Human Health-Banuelos, Lin and Yin (Eds.) London: Taylor and Francis, pp. 124-126.

199. Lindequist U, Niedermeyer THJ, Jülich WD. (2005). The pharmacological potential of mushrooms. *Evidence-based Complementary and Alternative Medicine*, 2:285–299.

200. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD 3rd,Crawford ED, Goodman GE, Claudio J, Winquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL Jr, Baker LH, Coltman CA

Jr.(2009). Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA*. 301: 39-51.

201. Liu Y, Chiba M, Inaba Y, Kondo M. (2002). Keshan disease: A review from the aspect of history and etiology. *Japanese Journal of Hygiene.* 56: 641-648.
202. Lobinski R, Edmonds J, Suzuki K, Uden P. (2000). Species-selective determination of selenium compounds in biological materials. *Pure and Applied Chemistry.*72: 447-461.
203. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 193: 265-275.
204. Luoma S.N, Presser TS. (2009). Emerging opportunities in management of selenium contamination. *Environmental Science and Technology.* 43: 8483-8487.
205. Macheix J, Fleuriet A. (1998). Phenolic acids in fruits. *In:* Rice-Evans CA, Packer L, (eds.) *Flavonoids in Health and Disease,* New York: Marcel Dekker, pp. 35-59.
206. Magnuson ML, Creed JT, Brockhoff CA. (1997). Speciation of selenium and arsenic compounds by capil- lary electrophoresis with hydrodynamically modified electro-osmotic flow and on-line reduction of selenium (VI) with hydride generation ICP-MS detection. *Analyst*. 122: 1057-1061.
207. Maiorino M, Ursini F. (2002). Oxidative stress, spermatogenesis and fertility. *Biological Chemistry*. 383: 591–597.
208. Mallikarjuna SE, Ranjini A, Haware DJ, Vijaylakshmi MR, Shashirekha MN, Rajarathnam S. (2013). Mineral composition of four edible mushrooms. *Journal of Chemistry*. DOI: 10.1155/2013/805284.
209. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. (1999). Nutrients in edible mushrooms: an inter-species comparative study. *Food Chemistry*. 65: 477-482.
210. Marian E. (1984). Introduction on environmental chemistry and global cycle of Cr, Ni, Co, Be, As, Cd and Se and their derivatives. *Toxicological and Environmental Chemistry* 8:9.
211. Maseko T, Howell K, Dunshea FR, Ng K. (2014). Selenium enriched *Agaricus bisporus* increases expression and activity of glutathione peroxidase-1 and expression of glutathione-2 in rat colon. *Food Chemistry*.146: 327-333.

212. Maseko T, Callahan DL, Dunshea FR, Doronila A, Kolev SD, Ng K. (2013). Chemical characterization and speciation of organic selenium in cultivated selenium-enriched *Agaricus bisporus. Food Chemistry*. 141: 3681-3687.

213. Masscheleyn PH, Delaune RD, Patrick WH. (1991). Biogeochemical behaviour of selenium in anoxic soils and sediments equilibrium thermodynamics approach. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering.* 26: 555–573.

214. Mattila P, Konko K, Eurola M, Pihlava JM, Astola J, Vahteristo L, Hietaniemi V, Kumpulainen J, Valtonen M, Piironen V. (2001). *Journal of Agricultural and Food Chemistry*. 49, 2343.

215. Mattila P, Sounpa K, Piironen V. (2000). Functional properties of edible mushrooms. *Journal of Nutrition*. 16: 7-8.

216. Mau JL, Lin HC, Song SF. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International.* 35: 519-526.

217. Mau JL, Chang CN, Huang S, Chen CC. (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa, Morchella esculenta* and *Termitomyces albuminosus*. *Food Chemistry*. 87: 111-118.

218. Mau JL, Chao GR. Wu KT. (2001). Antioxidant properties of methanolic exrtracts from several ear mushrooms. *Journal of Agricultural and Food Chemistry*. 49: 5461-5467.

219. Mau JL, Tsai SY, Tseng YH Huang SJ. (2005). Antioxidant properties of methanolic extracts of *Ganoderma tsugae*. Murrill. *LWT-Food Science and Techonology*. 93: 641-649.

220. Mayland HF, James JG, Panter KE, Sonderegger JL. (1989). Selenium in seleniferous environments. In: Jacob JW (ed.), *Selenium in Agriculture and the Environment*. SSSA Publication 23, Madison: SSSA, pp. 15–50.

221. Mayland HF. (1994). Selenium in plant and animal nutrition. In: Frankenberger WT, Benson S. (eds.), *Selenium in the Environment*. New York: Marcel Dekker, pp. 29−45.

222. McNaughton SA, Marks GC. (2002). Selenium content of Australian foods: A review of literature values. *Journal of Food Composition and Analysis.* 15: 169-182.

223. McNeal HF, Balistrieri LS. (1989). Geochemistry and occurrence of selenium: An overview, In: Jacob JW (ed.), *Selenium in Agriculture and the Environment*. SSSA Publication 23, Madison: SSSA, pp. 1-13

224. Mcsheehy S, Yang W, Pannier F, Szpunar J, Lobinski R, Auger J, Potin-Gautier M. (2000). Speciation analysis of selenium in garlic by two-dimensional high-performance liquid chromatography with parallel inductively coupled plasma mass spectrometric and electrospray tandem mass spectrometric detection. *Analytical Chimica Acta*. 421: 147-153.

225. McSheehy S, Yang L, Sturgeon R, Mester Z. (2005). Determination of methionine and selenomethionine in selenium-enriched yeast by species-specific isotope dilution with liquid chromatography−mass spectrometry and inductively coupled plasma mass spectrometry detection. *Analytical Chemistry*. 77: 344-349.

226. Medina D, Morrison DG. (1988) Current ideas on selenium as a chemoprevent agent. *Pathology and Immunopathology Research* 7: 187-199.

227. Melville DB, Horner WH, Otken CC, Ludwig ML. (1955). Studies on the origin of ergothioneine in animals. *Journal of Biological Chemistry*. 213: 61-68.

228. Michelot D, Siobud E, Dore JC, Viel C, Poirier F. (1998). Update on metal content profiles in mushrooms-toxicological implications and tentative approach to the mechanisms of bioaccumulation. *Toxicon.* 36: 1997–2012

229. Mihara H, Kurihara T, Yoshimura T, Esaki N. (2000). Kinetic and mutational studies of three NifS homologs from *Escherichia coli*: Mechanistic difference between L-cysteine desulfurase and L-selenocysteine lyase reactions. *Journal of Biochemistry* 127: 559-567.

230. Minotti G, Aust SD. (1987). The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. *Journal of Biological Chemistry*, 262: 1098-1104.

231. Montes-Bayón M, Díaz Molet MJ, Blanco González E, Sanz-Medel, A. (2006). Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (*Allium sativum* and *Brassica juncea*) and Se speciation by HPLC-ICP-MS. *Talanta*. 68:1287-1293.

232. Mosher BW, Duce RA. (1987). A global atmospheric selenium budget. *Journal of Geophysical Research: Atmosphere*. 92:13289–13298.

233. Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*.16: 55-63.

234. Mugesh G, Panda A, Singh HB, Punekar NS, Butcher RJ. (2001). Thiol-peroxidase activity of diaryl diselenides: A mechanistic study. *Journal of the American Chemical Society*.123: 839-50.

235. Munoz AHS, Kubachka K, Wrobel K, Corona JFG, Yathavakilla SKV, Caruso JA, Wrobel K. (2006). Se-enriched mycelia of *Pleurotus ostreatus*: Distribution of selenium in cell walls and cell membranes/cytosol. *Journal of Agricultural and Food Chemistry*. 54: 3440–3444.

236. Mutanen M. (1986). Bioavailability of selenium in mushrooms, *Boletus edulis*, to young women. *International Journal for Vitamin and Nutrition Research*. 56: 297–301.

237. National Academy of Science–National Research Council (NAS–NRC) (1976). *Selenium: Committee on Medical and Biological Effects of Environmental Pollutants*. NAS–NRC, Washington, DC.

238. Navarro-Alarcon M, Lopez-Martinez M.C. (2000). Essentiality of selenium in the human body: relationship with different diseases. *The Science of the Total Environment*. 249: 347-371.

239. Neal RH, Sposito G. (1989). Selenate adsorption on alluvial soils. *Soil Science Society of America Journal.* 53:70–74.

240. Neve J. (1996). Selenium as a risk factor for cardiovascular disease. *Journal of Cardiovascular Risk*, 3: 42-47.

241. Neve J. (1999). Combined selenium and iodine deficiency in Kashin-Beck osteoarthropathy. In: *STDA Bulletin*, Grimbergen: Selenium-Tellurium Development Association. pp. 1–3.

242. Nriagu JO. (1989). A global assessment of natural sources of atmospheric trace metals. *Nature*. 338: 47–49.

243. Nriagu JO, Pacyna JM. (1988). Quantitative assessment of worldwide contamination of air water and soils by trace metals. *Nature.* 333: 134–139.

244. Ogra Y, Ishiwata K, Encinar JR, Lobinski R, Suzuki KT. (2004). Speciation of selenium in selenium-enriched shiitake mushroom, *Lentinula edodes*. *Analytical and Bioanalytical Chemistry*, 379: 861–866.

245. Oke F, Aslim B. (2011). Protective effect of two edible mushrooms against oxidative cell damage and their phenolic composition. *Food Chemistry*. 128: 613-619.

246. Oldfield JE. (2006). Selenium: A historical perspective. In: Hatfield, DL, Berry MJ, Gladyshev VN (eds.), *Selenium: Its Molecular Biology and Role in Human Health* (2nd Ed.), New York: Springer, pp. 1-6.

247. Oldfield JE. (1999). *Selenium World Atlas*. Selenium–Tellurium Development Association, Grimbergen: STDA, pp 59.

248. Olson OE, Novacek EJ, Whitehead EI, Palmer IC. (1970). Investigations on selenium in wheat. *Phytochemistry* 9: 1181–1188.

249. Palacios I, Lozana M, DArrigo M, Rostagno MA, Martinez JA, Garcia-Lafuente A, Guillamon E, Villares A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*. 128: 674-678.

250. Papp LV, Lu J, Holmgren A, Khanna KK. (2007). From selenium to selenoproteins: Synthesis, identity, and their role in human health. *Antioxidants and Redox Signaling* 9:775–806.

251. Pedrero Z, Elvira D, Cámara C, Madrid Y. (2007). Selenium transformation studies during broccoli (*Brassica oleracea*) growing process by liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS). *Analytica Chimica Acta*. 596:251-256.

252. Peng A, Yang C, Rui H, Li H. (1992). Study on the pathogenic factors of Kashin-Beck disease. *Journal of Toxicology and Environmental Health.* 35: 79-90.

253. Peretz A, Siderova V, Neve J. (2001). Selenium supplementation in rheumatoid arthritis investigated in a double blind, placebo controlled trial. *Scandinavian Journal of Rheumatology*. 30: 208–212.

254. Pfeifer H, Conrad M, Roethlein D, Kyriakopoulos A, Brielmeier M, Bornkamm G, Behne D. (2001). Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. *Federation of American Societies for Experimental Biology Journal*. 15: 1236–1238.

255. Piepponen S, Pellinen M.J, Hattula T. (1984). The selenium content of mushrooms. In: Bratter P, Schrannel P. (eds.), In: *Trace Element - Analytical Chemistry in Medicine and Biology*. Berlin:Walter de Gruyter, pp. 159–166.

256. Prabhu KS, Zamamiri-Davis F, Stewart JB, Thompson JT, Sordillo LM, Reddy CC. (2002). Selenium deficiency increases the expression of inducible nitric oxide synthase in RAW 264.7 macrophages: role of nuclear factor-jB in up-regulation *Biochemistry Journal.* 366: 203-209.

257. Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SMN, Somasundaram R. (2006). Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural and Food Chemistry*. 54: 9764-9772.

258. Pyrzynska K. (1998). Speciation of selenium compounds. *Analytical Sciences*. 14: 479-483.

259. Quinche J-P. (1979). L"*Agaricus bitorquis*, un champignon accumulateur de mercure, de selenium at de cuivre. Revue suisse de viticulture, *d'arboriculture et d'horticulture*. 11: 189–192.

260. Quinche J-P. (1983). Les teneurs en sélénium de 95 espèces de champignons et de quelques terres, *Recherche Agronomie Suisse.* 22: 137–144.

261. Ragunathan R, Swaminathan K. (2003). Nutritional status of *Pleurotus spp.* grows on various agro-wastes. *Food Chemistry*. 80: 371- 377.

262. Rajarathnam S, Shashirekha MN, Bano Z. (1998). Biodegradative and biosynthetic capacities of mushrooms: Present and future strategies. *Critical Reviews in Biotechnology*. 18: 91–236.

263. Ravikovitch S, Margolin M. (1957). Selenium in soils and plants. *Agricultural Research Station, Rehovot.* 7: 41–52.

264. Rayman MP. (2000). The importance of selenium to human health. *Lancet*, 356: 233-241.

265. Rayman MP. (2002). The argument for increasing selenium intake. *Proceedings of the Nutrition Society.* 61: 203–215.

266. Rayman MP. (2004). The use of high-selenium yeast to raise selenium status: how does it measure up. *British Journal of Nutrition*. 92: 557-573.

267. Rayman MP. (2005). Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proceedings of the Nutrition Society*. 64: 527-542.

268. Rayman MP. (2008). Food-chain selenium and human health: emphasis on intake. *British Journal of Nutrition*, 100: 254-268.

269. Rayman MP, Angus F, Goenaga-Infante H. (2007). Bioavailability and speciation of selenium from selenium-enriched mushrooms. *Proceedings of Nutritional Society*. 66: 55A.

270. Rayman MP, Clark LC. (2000). Selenium in cancer prevention. In: Roussel AM, Favier A, Anderson RA (eds.), *Proceedings of the Tenth International Symposium on Trace Elements in Man and Animals*. New York: Plenum Press. pp. 575–580.

271. Rayman MP, Infante HG, Sargent M. (2008). Food-chain selenium and human health: Spotlight on speciation. *British Journal of Nutrition*, 100:238–253.

272. Recommended Dietary Allowances (RDA) (10th Ed.). 1989. National Academy of Sciences, National Academy Press, Washington DC, USA.

273. Reeves MA, Hoffmann PR. (2009). The human selenoproteome: recent insights into functions and regulation. *Cellular and Molecular Life Sciences*. 66:2457-2478.

274. Regan L. (1993). The design of metal binding sites in proteins. *Annual Review of Biophysics and Biomolecular Structures*. 22:257–87.

275. Reilly C. (1996). *Selenium in Food and Health*. N.York: Blackie Academic Professional. pp. 338.

276. Reily C. (2006). *Selenium in Food and Health*. (2nd ed.). New York: Springer.

277. Reis FS, Martins A, Barros L, Ferreira ICFR. (2012): Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between *in vivo* and *in vitro* samples. *Food and Chemical Toxicology*, 50: 1201-1207.

278. Rios JJ, Rosales MA, Blasco B, Cervilla LM, Romero L, Ruiz JM. (2008). Biofortification of Se and induction of the antioxidant capacity in lettuce plants. *Scientia Horticulture*, 116:248-255.

279. Rodrigo R, Castillo R, Carrasco R, Huerta P, Moreno M. (2005). Diminution of tissue lipid peroxidation in rats is related to the in vitro antioxidant capacity of wine. *Life Sciences.* 76: 889- 900.

280. Rodriguez MMJ, Rivero CV, Ballesta JR. (2005). Selenium distribution in topsoils and plants of semi-arid mediterranian environments. *Environmental Geochemistry and Health*. 27: 513-519.

281. Rosenfeld I, Beath OA. (1964). *Selenium: Geobotany, Biochemistry, Toxicity and Nutrition*. New York:Academic Press, pp. 411

282. Sabichi AL, Lee JJ, Taylor RJ, Thompson IM, Miles BJ, Tangen CM, Minasian LM, Pisters LL, Caton JR, Basler JW, Lerner SP, Menter DG, Marshall JR, Crawford ED, Lippman SM. (2006). Selenium accumulation in prostate tissue during a randomized, controlled short-term trial of l-selenomethionine: A Southwest Oncology Group study. *Clinical Cancer Research*, 12: 2178-2184.

283. Saitoh H, Feng W, Matsuzawa T, Ikekawa T. (1997). Antitumour activity of *Hypsizigus marmoreus*. II. Preventive effect against lung metastasis of Lewis lung carcinoma. *Yakugaku Zasshi*. 117: 1006–1010.

284. Sarikurkchu C, Tepe B, Semiz DK, Solak MH. (2010). Evaluation of metal concentration and antioxidant activity of three edible mushroom from Mugla. *Food and Chemical Toxicology*. 48: 1230-1233.

285. Savic M, Dubljanin B, Kostic M, Vasiljevic J, Niksic M. (2011). Accumulation of selenium from selenized yeast in the cultivated mushroom *Agaricus bisporus*. In: *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Biology and Mushroom Products*, France, pp. 135-139.

286. Sayato Y, Hasegawa T, Taniguchi S, Maeda H, Ozaki K, Narama I, Nakamuro K. (1993). Acute and subacute oral toxicity of selenocysteine in mice. *Japanese Journal of Toxicology and Environmental Health*. 39: 289-296.

287. Savic M, Andelkovic I, Duvnjak D, Matijasevic D, Avramovic A, Niksic M. (2012). The fungistatic activity of organic selenium and its application to the production of cultivated mushroom *Agaricus bisporus* and *Pleurotus sp*. *Archives of Biological Science Belgrade*. 64: 1455-1463.

288. Schrauzer GN. (1998). Selenium: present and future applications in medicine. In: *STDA Sixth International Symposium on Uses of Selenium and Tellurium*, Scottsdale, USA. Grimbergen, Belgium: SeleniumTellurium Development Association, pp. 37-40.

289. Schwarz K, Foltz CM. (1957). Selenium as an integral part of factor 3, against dietary liver necrosis degeneration. *Journal of American Chemical Society*. 79: 3292–3293.

290. Schweizer U, Brauer AU, Kohrle J, Nitsch R, Savaskan NE. (2004). Selenium and brain function: a poorly recognized liaison. *Brain Research Reviews*. 45: 164–178.

291. Seby F, Potin GM, Lespes G, Astruc M. (1997). Selenium speciation in soil after alkaline extraction. *Science of the Total Environment*. 207: 81-90

292. Severson RC, Gough LP. (1992). Selenium and sulphur relationship in alfalfa and soil under field conditions, San Joaquin Valley, California. *Journal of Environmental Quality*. 21: 353–358.

293. Shaheen SO, Sternejac, Thompson RL, Songhurst CE, Margetts, BM, Burney PGJ. (2001). Dietary antioxidants and asthma in adults. *American Journal of Respiratory and Critical Care Medicine*, 164:1823-1828.

294. Sharma N, Kumar A, Prakash R, Tejo Prakash N. (2007). Selenium accumulation and Se-induced Anti-oxidant activity in *Allium cepa. Environmental Informatics Archives,* 5: 328-333

295. Sharma N, Prakash R, Srivastava A, Sadana US, Acharya R, Tejo Prakash N, Reddy AVR. (2009). Profile of selenium in soil and crops in seleniferous area of Punjab, India by neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry*. 281:59-62.

296. Sharma S, Singh R. (1983). Selenium in soil, plant, and animal systems. *Critical Reviews in Environmental Control* 13:23-50.

297. Singh M, Kumar, P. (1976). Selenium distribution in soils of bio-clamatic zones of Haryana, India. *Journal of the Indian Society of Soil Science*. 24: 62-67.

298. Singleton VL, Rossi JA. (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *American Journal of Enology and Viticulture.* 16:144-158.

299. Slejkovec Z, Van Elteren JT, Woroniecka UD, Kroon K J, Falnoga I, Byrne AR. (2000). Preliminary study on the determination of selenium compounds in some selenium-accumulating mushrooms. *Biological Trace Element Research*. 75: 139–155.

300. Smith A, Cheung L, Botero S. (2011). Long-term selenium deficiency increases the pathogenicity of *Citrobacter rodentium* infection in mice. *Biological Trace Element Research.*144:965–982.

301. Song Z, Jia L, Feng X, Meng F, Deng P, Keming F, Liu X. (2009). Characteristics of Se-enriched mycelia by *Stropharia rugoso-annulata* and its antioxidant properties *in vivo*. *Biological Trace Element Research*. 131: 81-89.

302. Spallholz J E, Boylan LM, Rahman MM. (2004). Environmental hypothesis: Is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Science of the Total Environment*. 323: 21-32.

303. Spallholz JE, Boylan LM, Larsen HS. (1990). Advances in understanding selenium's role in the immune system. *Annals of the New York Academy of Sciences.* 587: 123–139.

304. Spolar MR, Schaffer EM, Beelman RB, Milner JA. (1999). Selenium-enriched *Agaricus bisporus* Mushrooms suppress 7,12-dimethlbenz[a]anthracene bioactivation in mammary tissue. *Cancer Letters*. 138: 145-150.

305. Stadlober M, Sager M, Irgolic KJ. (2001). Effects of selenate supplemented fertilization on selenium levels of cereals- Identification and quantification of selenium components by HPLC-ICP-MS. *Food Chemistry*.73: 357-366.

306. Stadtman, T. (1974) Selenium biochemistry. *Science*. 183: 915-922.

307. STDA, Selenium Tellurium Development Association, (2006). http://www.stda.net/se-te.htm.

308. Stefanka Z, Ipolyi I, Dernovics M, Fodor P. (2001). Comparison of sample preparation methods based on proteolitic enzymatic processes for Se-speciation of edible mushroom (*Agaricus bisporus*) samples. *Talanta*. 55: 437–447.

309. Steinbrenner H, Speckmann B, Pinto A, Sies H. (2011). High selenium intake and increased diabetes risk: experimental evidence for interplay between selenium and carbohydrate metabolism. *Journal of Clinical Biochemistry and Nutrition.* 48:40–45.

310. Stijve T, Besson R. (1976). Mercury, cadmium, lead and selenium of mushroom species belonging to the genus *Agaricus*. *Chemosphere.* 2: 151–158.

311. Stijve T, Cardinale E. (1974). Selenium and mercury content of some edible mushrooms. *Travaux De Chimie Alimentarie EtdHygeine* 65: 476–478.

312. Stijve T, Noorloos T, Byrne AR, Slejkovec Z, Goessler W. (1998). High selenium levels in edible *Albatrellus* mushrooms. *Deut Lebensm Rundsch*. 94: 275–279.

313. Stijve T. (1977). Selenium content of mushrooms, *Zeitschrift für Leibensmittel-Untersuchung und -Forschung* 164: 201–203.

314. Stranges S, Sieri S, Vinceti M, Grioni S, Guallar E, Laclaustra M, Muti P, Berrino F, Krogh VA. (2010), prospective study of dietary selenium intake and risk of type 2 diabetes. *BMC Public Health*, 10:564.

315. Sturchlerpierrat C, Carbon P, Krol A. (1995). Selenium and selenoproteins - An alternative reading of the genetic code. *Medicine Sciences*, 11: 1081-1088.

316. Surai P. (2006). *Selenium in Nutrition and Health*. Nottingham: Nottingham University Press.

317. Suzuki KT, Shiobara Y, Itoh M, Ohmichi, M. (1998). Selective uptake of selenite by red blood cells. *Analyst*. 123:63-67.

318. Suzuki T. (1965). A geochemical study of selenium in volcanic exhalation and sulphur deposits. II. On the behaviour of selenium and sulphur in volcanic exhalation and sulphur deposits. *Bulletin of Chemical Society of Japan*.38: 1940–1946.

319. Swanson CA, Patterson BH, Levander OA, Veillon C, Taylor PR, Helzlsloner K, Mcadam PA, Zech LA. (1991). Human [^{74}Se] selenomethionine metabolism: A kinetic model. *American Society for Clinical Nutrition*, 54:917-926.

320. Tamari Y, Ogawa H, Fukumoto Y, Tsuji H, Kusaka Y. (1990). Selenium content and its oxidation-state in igneous rocks, rock-forming minerals, and a reservoir sediment. *Bulletin of the Chemical Society of Japan*. 63:2631–2638.

321. Tan JA, Wang WY, Wang DC, Hou SF. (1994). Adsorption, volatilization, and speciation in different types of soils in China, In: Frankenberger Jr WT, Benson S, (eds.). *Selenium in Environment*. New York: Marcel Dekker, pp. 47-67.

322. Tapiero H, Townsend DM, Tew KD. (2003). The antioxidant role of selenium and seleno-compounds. *Biomedicine Pharmacotheraphy*. 57:134–144.

323. Taylor EW (1997) Selenium and viral diseases: Facts and hypotheses. *Journal of Orthomolecular Medicine.* 12: 227-239.

324. Teissedre PL, Waterhouse AL. (2000). Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oil varieties. *Journal of Agricultural and Food Chemistry.* 48: 3801-3805.

325. Tejo Prakash N, Sharma N, Prakash R, Nathaniel TN, Acharya R, Reddy AVR. (2010). Selenium fortification and pro/antioxidant responses in *Allium Cepa* (onion) cultivated in selenium supplemented soil. *Experimental Agriculture*. 46: 531-540.

326. Tham LX, Giang N, Hanh NTD. (2009). Study on selenium accumulation in Turley tail fungus (*Trametes versicolor*) by using instrumental neutron activation analysis. *Tap Chi Khoa Hoc Cong Nghe*. 47: 73-79.

327. Tokunaga T, Pickering I, Brown G. (1996). Selenium transformations in ponded sediments. *Soil Science Society of America Journal* 60:781-790.

328. Trbojevic IS, Ognjanovic BI, Dordevic NZ, Markovic SD, Stajin AS, Gavric JP, Saicic ZS. (2010). Effect of cisplatin on lipid peroxidation and the glutathione redox status in the liver of male rats: The protective role of selenium. *Archives of Biological Science-Belgrade*. 62: 75-82.

329. Trelease SF. (1945). Selenium in soils, plants and animals. *Soil Science*. 60: 125-132.

330. Turlo J, Gutkowska B, Herold F, Klimaszewska M, Suchockl P. (2010). Optimization of selenium-enriched mycelium of *Lentinula edodes (Berk.) pegler* as a food supplement. *Food Biotechnology*, 24:180–196.

331. US Department of Agriculture-Agricultural Research Service (USDA-ARS), 2008. *Nutrient Intakes from Food: Mean Amounts Consumed Per Individual One Day* (https://ars.usda.gov/)

332. Uden PC. (2002). Modern trend in speciation of selenium by hyphenated techniques. *Analytical Bioanalytical Chemistry*. 373:422-431.

333. Ulziijargal E, Mau J-L. (2011). Nutrient composition of culinary-medicinal mushroom fruiting bodies and mycelia. *International Journal of Medicinal Mushrooms*. 13: 343-349.

334. Vadhanavikit S, Ip C, Ganther HE. (1993). Metabolites of sodium selenite and methylated selenium compounds administered at cancer chemoprevention levels in the rat. *Xenobiotica.* 23: 731–745.

335. Van Campen DR, Glahn RP. (1999). Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Research.* 60: 93–113.

336. Vanderpas JB, Contempre B, Duale NL, Deckx H, Bebe N, Longombe AO, Thilly CH, Diplock AT, Dumont JE. (1993). Selenium deficiency mitigates hypothyroxinemia in iodine deficient subjects. *The American Journal of Clinical Nutrition*. 57: 271S–275S.

337. Veres Z, Kim IY, Scholz TD, Stadtman TC. (1994). Selenophosphate synthetase. Enzyme properties and catalytic reaction. *Journal of Biological Chemistry*. 269:10597-10603.

338. Vetchinkina E, Loshchinina E, Kursky V, Nikitina V. (2013). Reduction of organic and inorganic selenium compounds by the edible medicinal basidiomycete *Lentinula edodes*

and the accumulation of elemental selenium nanoparticles in the mycelium. *Journal of Microbiology*. 51: 829-835.

339. Vetter J. (2003). Chemical composition of fresh and conserved *Agaricus bisporus* mushroom. *European Food Research and Techonology*. 217: 10-12.

340. Vetter J, Hajdu C, Gyorfi J, Maszlaver P. (2005). Mineral composition of the cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. *Acta Alimentaria*. 34: 441–451.

341. Vetter J, Lelley J. (2004). Selenium level of the cultivated mushroom *Agaricus bisporus*. *Acta Alimentaria*, 33: 297–301.

342. Vinceti M, Wei ET, Malagoli C, Bergomi M, Vivoli G. (2001). Adverse health effects of selenium in humans. *Reviews on Environmental Health.* 16: 233–251.

343. Vinson JA, Dabbagh YA, Serry MM, Jang J. (1995). Plants flavonoids, especially tea flavanols, are powerful antioxidants using as in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry*. 43: 2800-2802.

344. Vogel VG, McPherson RS. (1989). Dietary epidemiology of colon cancer. *Hematology Oncology Clinics of North America*. 3:35-63.

345. Vonderheide AP, Wrobel K, Kannamkumarath SS, B Hymer C , Montes-Bayón M , de Leon CP, Caruso JA. (2002). Characterization of selenium species in Brazil Nuts by HPLC−ICP-MS and ES-MS. *Journal of Agricultural and Food Chemistry*. 50: 5722-5728.

346. Vunta H, Belda BJ, Arner RJ, Reddy CC, Heuvel JP, Prabhu KS. (2008). Selenium attenuates pro-inflammatory gene expression in macrophages. *Molecular Nutrition and Food Reserach.* 52:1316–1323.

347. Vunta H, Davis F, Palempalli UD, Bhat D, Arner RJ, Thompson JT, Peterson DG, Reddy CC, Prabhu KS. (2007). The anti-inflammatory effects of selenium are mediated through 15-deoxy-Delta12, 14-prostaglandin J2 in macrophages. *Journal of Biological Chemistry*. 282: 17964–17973.

348. Wang C, Wang H, Luo J, Hu Y, Wei L, Duan M, He H. (2009). Selenium deficiency impairs host innate immune response and induces susceptibility to *Listeria monocytogenes* infection. *BMC Immunology*.10:55.

349. Wang XDC, Jiang H, Chen Z, Shi H, Li J. (2005). The Effect of the Se to the Fruiting-body of *Pleurotus ostreatus* and Nutrition Composition. *Food Science.* 26: 91-95.

350. Wasser SP, Weis AL. (1999). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives. *International journal of Medicinal Mushrooms*. 1:31–62.

351. Weiss HV, Koide M, Goldberg ED. (1971). Selenium and S in a Greenland ice sheet: Relation to fossil fuel combustion. *Science*. 172: 261–263.

352. Welch RM, Graham RD. (2005). Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. *Journal of Trace Elements in Medicine and Biology*. 18: 299–307.

353. Welsh SO, Holden JM, Wolf WR, Levander OA. (1981). Selenium in self-selected diets of Maryland residents. *Journal of the American Dietetic Association*. 79:277–285.

354. Werner AR, Beelman RB. (2002). Growing high-selenium edible and medicinal button mushrooms (*Agaricus bisporus* (J. Lge) Imbach) as ingredients for functional foods or dietary supplements. *International Journal of Medicinal Mushrooms*. 4:167-171.

355. Whanger PD. (2004). Selenium and its relationship to cancer: An update. *British Journal of Nutrition.* 91: 11–28.

356. Whanger PD. (2002). Selenocompounds in plants and animals and their biological significance. *The Journal of the American College of Nutrition.* 21: 223-232.

357. White PJ, Bowen HC, Parmaguru P, Fritz M, Spracklen WP, Spiby RE, Meacham MC, Mead A, Harriman M, Trueman LJ, Smith BM, Thomas B, Broadley MR (2004) Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. *Journal of Experimental Botany*. 55:1927–1937.

358. Wilber CG. (1980). Toxicology of selenium: A review. *Clinical Toxicology.* 17:171–230.

359. Wong JY, Chye FY. (2009). Antioxidant Properties of Selected Tropical Wild Edible Mushrooms. *Journal of Food Composition and Analysis*. 22: 269-277.

360. World Health Organization (WHO). (1987). *Selenium. A Report of the International Programme on Chemical Safety*. Environmental Health Criteria no. 58. Geneva: WHO.

361. World Health Organization (WHO). (2004). Vitamin and mineral requirements in human nutrition. In: *Report of a Joint FAO-WHO Expert Consultation*, Bangkok, Thailand. Switzerland: WHO. pp. 194–216.

362. Wright JS, Johnson ER, Dilabio GA. (2001). Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. *Journal of American Chemical Society*. 123: 1173-1183.

363. Wu L, Banuelos G, Guo X. (2000). Changes of soil and plant tissue selenium status in anupland grassland contaminated by selenium-rich agricultural drainage sediment after ten years transformed from a wetland habitat. *Exotoxicology and Environmental Safety*, 47: 201-209.

364. Wu L, Guo X, Banuelos GS. (2003). Selenium and sulfur accumulation and soil selenium dissipation in planting of four herbaceous plant species in soil contaminated with drainage sediment rich in both selenium and sulfur. *International Journal of Phytoremediation.* 5:24–40.

365. Wu L. (2004). Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. *Ecotoxicology and Environmental Safety.* 57: 257-269.

366. Wuilloud RG, Kannamkumarath SS, Caruso JA. (2004). Multielemental speciation analysis of fungi porcini (*Boletus edulis*) mushroom by size exclusion liquid chromatography with sequential on-line UV-ICP-MS detection. *Journal of Agricultural Food Chemistry.* 52:1315-1322.

367. Xia Y, Hill KE, Byrne DW, Xu J, Burk RF. (2005). Effectiveness of selenium supplements in a low-selenium area of China. *American Journal of Clinical Nutrition*. 81: 829–834.

368. Ximenez-Embun P, Alonso I, Madrid AY, Caa Mara C. (2004). Establishment of selenium uptake and species distribution in lupine, Indian mustard, and sunflower plants. *Journal of Agricultural Food Chemistry.* 52: 832-838.

369. Xu J, Yang F, An X, Hu Q. (2007). Anticarcinogenic activity of selenium-enriched green tea extracts *in vivo*. *Journal of Agricultural and Food Chemistry*. 55: 5349- 5353.

370. Yamaguchi R, Tatsumi MA, Kato K, Yoshitimitsu U. (1988). Effect of metal salts and fructose on the autoxidation of s-methyl linoleate in emulsions. *Agricultural and Biological Chemistry*. 52: 849-850.

371. Yamashita Y, Yabu T, Yamashita M. (2010). Discovery of the strong antioxidant selenoneine in tuna and selenium redox metabolism. *Journal of Biological Chemistry 285*: 18134-18138.

372. Yamashita Y, Yamashita M, Yabu T. (2011). *Selenium Containing Compounds*. US Patent # US 2011/0178018 A1.

373. Yan H, Chang H. (2012). Antioxidant and antitumor activities of selenium and zinc enriched oyster mushroom in mice. *Biological Trace Element Research*. DOI 10.1007/s 12011-012-9454-1.

374. Yang F. (2006). Keshan disease and mitochondrial cardiomyopathy. *Science in China Series C: Life Sciences,* 49: 513-518.

375. Yang FY, Lin ZH, Xing JR, Li SG, Yang J, San S, Wu L. (1994). Selenium deficiency is a necessary but not sufficient factor required for the pathogenesis of Keshan disease. *Journal of Clinical Biochemistry and Nutrition.* 16: 101–110.

376. Yang G, Wang S, Zhou R, Sun S. (1983). Endemic selenium intoxication of humans in China. *American Journal of Clinical Nutrition*. 37: 872–881.

377. Yang JH, Lin HC, Mau JL. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry*. 77: 229-235.

378. Yen GC, Chen HY. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry.* 43: 27-32.

379. Yoshida M, Sugihara S, Inoue Y, Chihara Y, Kondo M, Miyamotom S, Sukcharoen B. (2005). Composition of chemical species of selenium contained in selenium-enriched shiitake mushroom and vegetables determined by high performance liquid chromatography with inductively coupled plasma mass spectrometry. *Journal of Nutritional Science and Vitaminology*. 51:194–199.

380. Yu MW, Horng IS, Chiang YC, Liaw YC, Chen CJ. (1999).Plasma selenium levels and the risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. *American Journal of Epidemiology.* 150: 367-374.

381. Yu SY, Zhu YJ, Li WG. (1997). Protective role of selenium against hepatitis B virus and preliminary liver cancer in Qidong. *Biological Trace Element Research.* 56:117-124.

382. Yudovick YE, Ketris MP. (2006). Selenium in coal. *International Journal of Coal Geology*. 67: 112-126.

383. Yue W, Zhang C, Shi L, Ren Y, Jiang Y, Kleemann DO. (2009). Effect of supplemental selenomethioneine on growth performance and serum antioxidant status in Taihang black goats. *Asian-Australasian journal of Animal Science*. 22: 365-370.

384. Zhao L, Zhao G, Zhao Z, Chen P, Tong J, Hu X. (2004). Selenium distribution in a Se enriched mushroom species of the genus *Ganoderma*. *Journal of Agricultural and Food Chemistry*. 52: 3954–3959.

385. Zhu YG, Pilot-Smits EA, Zhao FJ, Williams PN, Mehrang AA. (2009). Selenium in higher plants: understanding mechanism for biofortification and phytoremediation. *Trends in Plant Sciences*.14: 436-442.

386. Zhuo H, Smith AH, Steinmaus C. (2004). Selenium and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. *Cancer Epidemiology, Biomarkers and Prevention*.13:771-778.

387. Zingaro R A, Cooper WC. (1974). *Selenium,* New York: Van-Nostrand Reinhold, pp. 835

388. Zinoni F, Birkmann A, Leinfelder W, Bock A. (1987). Cotranslational insertion of selenocysteine into formate dehydrogenase from *Escherichia coli* directed by a UGA codon. *Proceedings of the National Academy of Sciences (USA)*. 84: 3156-3160.

389. Zoller WH, Gladney ES, Duce RA. (1974). Atmospheric concentrations and sources of trace metals apt the South Pole. *Science*. 183:198-200.

List of Contributions

Publications:

1. Bhatia P, Aureli F, Amato M, Prakash R, Cameotra SS, Tejo Prakash N, Cubadda F. (2013). Selenium bioaccessibility and speciation in biofortified *Pleurotus* mushrooms grown on selenium-rich agricultural residues. *Food Chemistry.* 140: 225-230.

2. Bhatia P, Prakash R, Tejo Prakash N. (2013). Selenium uptake by edible oyster mushrooms (*Pleurotus* spp) from selenium hyperaccumulated wheat straw. *Journal of Nutrition Science and Vitaminol*ogy. 59: 69-72.

3. Bhatia P, Bansal C, Prakash R, Tejo Prakash N. (2014). Selenium uptake and associated anti-oxidant properties in *Pleurotus fossulatus* cultivated on wheat straw from seleniferous fields. *Acta alimentaria*. 43:280-287.

Conference presentations:

1. Bhatia P, Sodhi HS, Prakash R, Tejo Prakash N. (2013). Uptake of selenium and associated anti-oxidant properties in *Volvariella volvacea* cultivated on agricultural residues from seleniferous fields. *Indian Mushroom Conference (IMC),* PAU, Ludhiana. pp. 23. (**Awarded with best poster presentation**).

2. Bhatia P, Prakash R, Tejo Prakash N. (2013). Selenium induced anti-oxidant activity in *Agaricus bisporus* cultivated on Se-hyperaccumulated agri-residues. *National Conference on Innovative Molecules for Sustainable Future (NCIMSF),* Thapar University, Patiala. pp.83.

3. Bhatia P, Aulakh SS, Prakash R, Cameotra SS, Tejo Prakash N. (2011). Selenium fortification from Se rich Wheat Straw to Edible Mushrooms. *4th International Conference on Trace Elements in Foods (TEF 4), A*berdeen, UK . pp. 40.

Food Chemistry 140 (2013) 225–230

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Selenium bioaccessibility and speciation in biofortified *Pleurotus* mushrooms grown on selenium-rich agricultural residues

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ARTICLE INFO

Article history:
Received 18 April 2012
Received in revised form 21 December 2012
Accepted 12 February 2013
Available online 24 February 2013

Keywords:
Selenium
Speciation
HPLC-ICP-MS
In vitro gastrointestinal digestion
Fortification
Oyster mushrooms
Wheat straw

ABSTRACT

Cultivation of saprophytic fungi on selenium-rich substrates can be an effective means to produce selenium-fortified food. *Pleurotus florida*, an edible species of oyster mushrooms, was grown on wheat straw from the seleniferous belt of Punjab (India) and its potential to mobilize and accumulate selenium from the growth substrate was studied. Selenium concentration in biofortified mushrooms was 800 times higher compared with control samples grown on wheat straw from non selenium-rich areas (141 *vs* 0.17 μg Se g^{-1} dry weight). Seventy-five percent of the selenium was extracted after *in vitro* simulated gastrointestinal digestion and investigation of the selenium molecular fractions by size exclusion HPLC-ICP-MS revealed that proteins and any other high molecular weight selenium-containing molecule were hydrolyzed to peptides and low molecular weight selenocompounds. Analysis of the gastrointestinal hydrolysates by anion exchange HPLC-ICP-MS showed that the bioaccessible selenium was mainly present as selenomethionine, a good bioavailable source of selenium, which accounted for 73% of the sum of the detected species.

This study demonstrates the feasibility of producing selenium-biofortified edible mushrooms using selenium-rich agricultural by-products as growth substrates. The proposed approach can be used to evaluate whether selenium-contaminated plant waste materials harvested from high-selenium areas may be used to produce selenium-biofortified edible mushrooms based on the concentration, bioaccessibility and speciation of selenium in the mushrooms.

1. Introduction

Selenium (Se) is an essential micronutrient for humans and animals, involved in functioning at the catalytic center of several selenoproteins (Rayman, 2000). It is the only trace element to be specified in the genetic code as selenocysteine (SeCys), which has been recognised as the 21st amino acid having its own codon and SeCys-specific biosynthetic and insertion machinery (Rayman, 2002).

Although Se is nearly ubiquitous in soils, it exists mainly in insoluble forms in high-iron and organic matter, low pH and certain leached soils, and hence is often of limited availability to plants (Fordyce, Zhang, Green, & Liu, 2000; Johnson, Ge, Green, & Liu, 2000; Spadoni et al., 2007). Consequently, it is often supplied by plants to animals and humans at levels too low for optimum health. Se deficiency and sub-optimality are associated with health disorders including oxidative stress-related conditions (*e.g.*, cardiovascular disease, various inflammatory syndromes), thyroid dysfunction, reduced fertility and immune functions, increased susceptibility to viral infection and increased risk of cancers (Rayman, 2000, 2002). Selenium deficiency probably affects at least a billion people (Combs, 2001). Increasing population intake in regions where Se is in short supply in the food chain may result in improvement in public health and health cost savings.

Several strategies to raise Se intake may be employed, including education to increase consumption of higher-Se foods, individual supplementation, food fortification, supplementation of livestock, Se fertilisation of crops, and plant breeding for enhanced Se accumulation (Combs, 2001; Rayman, 2008). In order to select the best solution, it must be considered that there are adverse consequences for health of both deficient and excessive intake and that optimal intake at the individual level depends on a large number of factors, such as the functions of Se most relevant to a particular

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0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.02.054

disease state, the Se species most prominent in the Se source, the health condition being considered, the adequacy of intake of other nutrients, the presence of additional stressors, and lastly whether the ability to make selenoproteins may be compromised (Rayman, 2008).

Mushrooms are interesting food items for the formulation of a healthy diet because they are poor in calories and in fat but rich in proteins, minerals and dietary fibre (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999; Manzi & Pizzoferrato, 2000). Dietary fibre includes components of fungal cell walls such as chitin, other hemi-celluloses, mannans and beta glucans, which are supposed to play a key role in some healthy properties of mushrooms, such as enhancement of macrophage function and host resistance to many bacterial, viral, fungal and parasitic infections, activation of a non-specific immune stimulation, reduction of blood cholesterol and blood glucose levels (Cheung, 2009). Although not plants, mushrooms are generally grouped with vegetables for dietary purposes. Se content of mushrooms is generally higher than that of most vegetables (Rayman, Infante, & Sargent, 2008) but it is very variable. Apart from the bioavailable concentrations in soil, the amount of selenium found in wild edible mushrooms is dependent on the species and the stage of maturity; for cultivated species the substrates used for growth are important (Kalac, 2009). Selenium concentration in the fruiting body of popular edible mushrooms covers over two orders of magnitude and ranges <1–20 μg Se g^{-1} dw, with the highest values found in the species of the genus *Boletus* (Falandysz, 2008). Selenium-fortified mushrooms cultivated on substrates supplemented with Se, usually as inorganic salt, have been proposed as Se-enriched functional foods (Falandysz, 2008). On the negative side, earlier studies reported Se from mushrooms to have a low bioavailability in rat (Chansler, Mutanen, Morris, & Levander, 1986) and man (Mutanen, 1986). On the contrary, recent work on Se-enriched mushrooms showed a good bioavailability *in vivo* (da Silva et al., 2010; Rayman, Angus, & Goenaga-Infante, 2007). Furthermore, Se-enriched *Agaricus bisporus* has been shown to be effective for the reduction of 7,12-dimethylbenz(a)anthracene (DMBA)-induced anti-3,4-dihydrodiol-1,2-epoxide-deoxyguanosine adducts in rats (Spolar, Schaffer, Beelman, & Milner, 1999). The divergent results may be explained by differences in chemical speciation, which affect the bioavailability and fate of Se in the body (Rayman et al., 2008). In fact, the studies carried out so far suggest that Se speciation changes from a mushroom species to another and may be influenced from the chemical species in the growth substrate as well (Table 1).

In the present study, Se-rich agricultural-residues from fields located in the seleniferous belt of Punjab (India) (Dhillon & Dhillon, 2009) were used as substrates for the cultivation of an edible species of oyster mushrooms (*Pleurotus* genus). The ability of the selected species to mobilize and accumulate Se from the wheat straw used as growth substrate was studied. The bioaccessibility and speciation of Se in the mushrooms were investigated by *in vitro* simulated gastrointestinal digestion and HPLC-ICP-MS.

2. Materials and methods

2.1. Instrumentation

Chromatographic separations were performed using an HPLC system consisting of a Perkin-Elmer Series 200 LC binary pump, an autosampler, and a column thermostat. The outlet of the HPLC column was directly connected via PEEK capillary tubing to the nebulizer of the Elan DRC II ICP-MS (Perkin-Elmer-Sciex, USA), which served as the Se specific detector both for total and speciation analysis. Instrumental settings are in Table 2. Chromatographic data were collected, stored, and processed using the Perkin-Elmer software Chromera. For the calibration of the size exclusion chromatography column, the chromatographic apparatus was connected to a Perkin-Elmer diode array detector operating at 280 nm.

2.2. Reagents and standards

Deionized water obtained by a Milli-Q Element System (Millipore, France) was used throughout the work. Nitric acid 68% v/v (Carlo Erba Reagenti, Italy) and hydrogen peroxide 31% v/v (Merck KGaA, Germany) both ultrapure grade were used for oxidative digestion of samples and gastrointestinal hydrolysates.

Calibrants and the internal standard solution (rhodium) used for total selenium measurements were obtained from standard certified solutions with a content of 1 mg mL^{-1} (High Purity Standard, USA), by dilution with acidified (HNO_3) deionized water as necessary. For speciation analysis, 1 mg mL^{-1} stock solutions, expressed as selenium, were prepared by dissolving in water adequate amounts of selenious acid [Se(IV)], selenic acid [Se(VI)], seleno-L-methionine (Sigma–Aldrich, USA). Standard stock solutions were stored at 4 °C and exact concentrations were ascertained by ICP-MS analysis. The purity of the standards was checked by HPLC-ICP-MS and no species interconversion was found.

The chromatographic mobile phases were prepared by dissolving appropriate amounts of analytical grade triethylamine (TEA-Sigma–Aldrich, USA), acetic acid 99% (Baker Instra Analyzed, Avantor Material, The Netherlands), and ammonium acetate (Merck KgaA, Germany) in water. Mobile phases were filtered through a Millipore Express Plus 0.22 μm membrane.

Blue dextran (200 kDa), albumin (66 kDa), carbonic anhydrase (29 kDa), cytochrome c (12.4 kDa), aprotinin (6.5 kDa), vitamin B12 (1.3 kDa) and SeMet (0.2 kDa) used as molecular weight calibrants in size exclusion chromatography were from Sigma (Sigma–Aldrich, USA).

Porcine enzymes (pepsin, pancreatin), α-amylase from *Bacillus subtilis*, sodium chloride, and bile salts were supplied by Sigma (Sigma–Aldrich, USA). Ultrapure grade hydrochloric acid 37% v/v (Carlo Erba Reagenti, Italy) and sodium hydrogen carbonate (Sigma–Aldrich, USA) were used to adjust pH during the simulated gastrointestinal digestion. 2-Merctaptoethanol 99% was from Sigma (Sigma–Aldrich, USA).

2.3. Procedures

2.3.1. Se biofortification of edible mushrooms

2.3.1.1. Culture collection. A pure culture of *Pleurotus florida* (Mont.) was provided from Punjab Agricultural University, Ludhiana (India). The propagation of the main culture was done on potato dextrose agar (PDA) medium. PDA plates were inoculated with a mycelium plug (6 mm in diameter) of an actively growing part of the colony and incubated at 25 °C in the dark for 7 days.

2.3.1.2. Spawn preparation. Selenium-rich wheat grain from the seleniferous belt of Punjab (India) was used for spawn production. The grains were washed with water and boiled for 30 min until they softened. Boiled grains were drained, supplemented with 2% calcium sulphate and 0.5% calcium carbonate and then mixed manually. Aliquots of 250 g were placed in glucose bottles and sterilized in autoclave at 121 °C for 15 min. After cooling, each bottle was inoculated with two agar disks of 6 mm diameter containing mycelium, and incubated at 25 °C in full darkness for 2 weeks.

2.3.1.3. Conditions of cultivation. Wheat straw harvested from the same selenium-rich site as above was used as substrate for mushroom cultivation. Wheat straw was cut into small pieces

Table 1
Summary of Se-species distribution in mushrooms.

Mushroom species	Origin	Extraction	Analytical technique[a]	Se-species[b]	Ref.
Albatrellus pes-caprae Boletus edulis	Wild grown	Chemical; enzymatic	HPLC-NAA and AFS	Se(IV); SeMet; $SeCys_2$; unknown compounds	Slejkovec et al. (2000)
Agaricus bisporus	Enriched	Chemical; enzymatic	HPLC-AFS	Se(IV); $SeCys_2$	Stefanka et al. (2001)
Agaricus bisporus	Enriched	Enzymatic	HPLC-HG-AFS	$SeCys_2$; Se(IV); SeMet	Dernovics, Stefanka, and Fodor (2002)
Boletus edulis	Wild grown	Chemical	HPLC-ICP-MS	LMW (<1.35–20 kDa); HMW (~50 kDa)	Wuilloud, Kannamkumarath, and Caruso (2004)
Boletus edulis	Wild grown	Chemical; enzymatic	HPLC-ICP-MS	$SeCys_2$; SeMet; MeSeCys; unknown compounds	Wilburn, Vonderheide, Soman, and Caruso (2004)
Lentinula edodes	Enriched	Chemical; enzymatic	HPLC-ICP-MS HPLC–ESI-MS	SeMet	Ogra, Ishiwata, Encinar, Lobinski, and Suzuki (2004)
Ganoderma lucidum	Enriched	Chemical	HPLC-AFS	SeMet	Zhao et al. (2004)
Macrolepiota procera; Lepista luscina; Boletus luridus	Wild grown	Chemical	HPLC-ICP-MS	SeMet; unknown compounds	Diaz Huerta, Fernandez Sanchez, and Sanz-Medel (2005)
Agaricus spp.	Enriched; cultivated	Chemical; enzymatic	HPLC-ICP-MS	SeMet; unknown compounds	Diaz Huerta, Fernandez Sanchez, and Sanz-Medel (2006)
Lentinula edodes	Enriched	Chemical; enzymatic	HPLC-ICP-MS	SeMet; Se(IV); unknown compounds	Yoshida et al. (2005)
Agaricus bisporus; Lentinula edodes	Enriched	Chemical; enzymatic	HPLC-ICP-MS	Se(IV); $SeCys_2$; MeSeCys; SeMet; unkown compounds	Gergely, Kubachka, Mounicou, Fodor, and Caruso (2006)

[a] NAA neutron activation analysis; AFS atomic fluorescence spectrometry; HG hydride generation; ICP-MS inductively coupled plasma mass spectrometry; HPLC high performance liquid chromatoghraphy; ESI-MS electrospray ionisation mass spectrometry.
[b] Identified species, listed in decreasing order of concentration. LMW low molecular weight; HMW high molecular weight.

and dipped in 1.5% formaldehyde until reaching a humidity of 70–75% (~24 h). After disinfection and softening, the straw was allowed to air dry before inoculation with *P. florida* spawn. Following inoculation, the straw was filled in plastic bags (3.0–4.0 kg each) and incubated at 25 °C for about 14 days. Humidity was maintained by spraying water twice a day. After 14 days of mycelial growth, the bags were torn and the bound matrix (straw and mycelium) was hung again under similar conditions for the appearance and growth of the fruiting bodies of mushrooms. On maturity (about 30 days after spawning), fruiting bodies were collected, weighed and dried at 40 °C in an oven for near complete dehydration (8–9% residual humidity). An identical procedure was followed for the cultivation of control mushrooms, with 'normal' (*i.e.*, not Se naturally-enriched) wheat straw as the growth substrate.

2.3.2. In vitro simulated gastrointestinal digestion

The *in vitro* enzymolysis procedure simulating human gastrointestinal digestion was carried out in triplicate. In parallel, procedural blanks were run to check the presence of Se in the reagents. Mushroom samples (0.5 g) were incubated with 5 mL of gastric juice (1% w/v pepsin in 0.15 M NaCl, adjusted to pH = 2 with HCl 37% v/v) and, after 1 min of vigorous shaking for initial degassing, were placed in a mixing water bath (GFL 1083, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at 37 °C for 4 h (Crews et al., 1996). Then, samples were adjusted to pH 6.8 with $NaHCO_3$ 2 M. After adding 5 mL of intestinal juice (3% w/v pancreatin, 1.5% w/v amylase, 1% w/v bile salts in 0.15 M NaCl), samples were vigorously shaken for 1 min, degassed and further incubated for 4 h at 37 °C under gentle shaking. Samples were then centrifuged at 8000*g* and 4 °C for 15 min, the supernatant collected, filtered through 0.45 μm membranes, divided into aliquots and stored at −80 °C until analysis.

2.3.3. Se analysis

2.3.3.1. Total Se analysis. Total selenium was measured in the cultivation substrates (*i.e.*, wheat grain and straw) and in mushrooms. Microwave digestion was performed by means of a Milestone Ethos Pro labstation (FKV, Italy) with quartz closed vessels, equipped with an infrared temperature control system. Approximately 0.3 g of sample were submitted in triplicate to 1 h pre-mineralization with 3 mL HNO_3 and then added with 1.5 mL of H_2O_2. Microwave irradiation was performed with temperature control and automatic continuous adjustment of power output, and the programme was as follows: 37 min ramp to 180 °C; 15 min at 180 °C.

Total Se liberated by gastrointestinal digestion was also determined by ICP-MS. Aliquots of extracts (2 mL) were added with 0.5 mL HNO_3 and 0.5 mL H_2O_2, and submitted to the same digestion procedure as above.

Total Se measurements were carried out in the Dynamic Reaction Cell mode using Rh as internal standard, the method of standard additions for quantification and the operating conditions listed in Table 2. The certified reference material NIST Durum Wheat Flour 8436 was included in each analytical batch for quality control. The accuracy of total Se determinations as assessed through NIST 8436 analysis was satisfactory; the certified value was 1.1 ± 0.2 μg Se g^{-1} dw and a trueness of 91% and precision of 3% were obtained.

2.3.3.2. Se speciation analysis. Se species liberated by simulated gastrointestinal digestion were characterised by size exclusion HPLC-ICP-MS and anion exchange HPLC-ICP-MS. Se species in extracts were identified by retention time matching with the standard substances spiked to the sample extracts. Quantitative calculations were based on peak areas. Measurements were carried out in standard mode with the operating conditions listed in Table 2.

3. Results and discussion

3.1. Se accumulation in mushrooms

Mushrooms cultivated on naturally Se-enriched substrates did not differ from controls in terms of yield (expressed as fresh mushroom weight per bag), biological efficiency (ratio of fresh mushroom weight to substrate dry weight expressed as a percentage), and production length (number of days elapsed from inoculation to harvest).

Selenium concentrations in the boiled wheat grain used for spawn production, in wheat straw used as growth substrate and in the mushrooms are shown in Table 3. Se levels in unenriched substrates are moderately low (Oldfield, 1999; Spadoni et al.,

Table 2
Instrumental operating conditions.

ICP-MS settings	
RF power	1.4 kW
Nebulizer gas flow rate, Ar	1.04 L min^{-1}
Lens voltage	9.0 V
DRC gas flow rate, CH_4	0.7 mL min^{-1}
Rejection parameter q (RPq)	0.45
Analytical masses	^{80}Se (total Se/DRC mode), ^{77}Se, ^{82}Se (Se speciation/ standard mode)
Chromatographic conditions	
Size exclusion chromatography	
Column	Superdex 75 10/300 GL (GE Healthcare)
Mobile phase	Ammonium acetate 100 mM, pH 7.5
Flow rate	0.5 mL min^{-1}
Injection volume	75 μL
Isocratic elution	0–60 min
Temperature	23 °C
Anion exchange chromatography	
Column	Hamilton PRP-X100 (250 mm × 4.1 mm, 5 μm) equipped with guard column
Mobile phase	(A) Acetic acid 20 mM, triethylamine 10 mM; (B) acetic acid 200 mM, triethylamine 100 mM
Flow rate	1 mL min^{-1}
Injection volume	100 μL
Gradient elution	0–5 min: 100% A; 5–30 min: 0–100% B; 30–40 min: 100% B; 40–41 min: 0–100% A; 41–45 min: 100% A
Temperature	23 °C

2007) and the resulting concentrations in the mushroom fruiting bodies are in the typical range of wild and cultivated *Pleurotus* species, *i.e.*, 0.12–3.4 μg Se g^{-1} dw (Costa-Silva, Marques, Matos, Barros, & Nunes, 2011; da Silva et al., 2012; Diaz-Alarcon, Navarro-Alarcon, Lopez-Garcia de la Serrana, & Lopez-Martinez, 1994; EuroFIR, 2012; Mattila et al., 2001; Vetter et al., 2005). This is very similar to the Se concentration range of the widely cultivated *A. bisporus*, *i.e.*, 0.2–3.6 μg Se g^{-1} dw (Falandysz, 2008; Kalac, 2010) and much lower than that of *Boletus edulis*, which is abundant in Se and can contain up to 70 μg Se g^{-1} dw (Falandysz, 2008).

Selenium concentrations in the substrates used to cultivate Se-enriched mushrooms are in the range of values we found for the same matrices from the identical area (Cubadda et al., 2010). *P. florida* grown on these substrates readily mobilized and accumulated the Se contained therein and the fruiting bodies exhibited Se concentrations ~800 times higher than control samples, *i.e.*, 141 *vs* 0.17 μg Se g^{-1} dw (Table 3). The ability of other *Pleurotus* species to absorb Se inorganic salts from the growth substrate and accumulate Se in uncharacterized form in the mycelium or in the fruiting bodies has been already reported (da Silva et al., 2012; Estrada, Lee, Beelman, Jimenez-Gasco, & Royse, 2009; Wang et al., 2005). In this study, it is shown that *P. florida* is able to mobilize Se from an organic substrate and concentrate it in the fruiting body at levels almost identical to those found in *A. bisporus* grown on compost supplemented with selenite (Stefanka, Ipolyi, Dernovics, & Fodor, 2001).

Expressed on a fresh weight basis, the Se concentration obtained in this study in Se-biofortified *P. florida* is 11.3 μg Se g^{-1}.

Table 3
Total Se concentrations expressed as μg Se g^{-1} dw ($n = 3$).

Sample type	Boiled wheat grain	Wheat straw	Mushrooms
Control	0.025 ± 0.001	0.026 ± 0.001	0.17 ± 0.01
Se-enriched	93.2 ± 0.8	27.9 ± 0.1	141 ± 2

Assuming negligible losses due to cooking, consumption of 5–7 g of mushrooms is sufficient to meet the recommended dietary allowance/reference nutrient intake (RDA/RNI) for adults, which is set to 55–75 μg Se/d depending on the country (CMAFP, 1991; EURRECA, 2012; FNB, 2000; SINU, 1996).

It has to be highlighted that the purpose of the present study was to explore the feasibility of producing Se-biofortified edible mushrooms using Se-rich agricultural-residues as growth substrates at the laboratory scale and to characterise the bioaccessibility and speciation of the Se in mushrooms. Depending on the intended use (*e.g.*, as functional food, as ingredient in the formulation of nutraceuticals, as Se dietary supplement) the production process can be adapted in order to achieve optimal Se concentration in the mushrooms by adjusting the Se levels in the substrates, which can be done, *e.g.*, by mixing wheat straw from Se-rich areas and areas with background concentrations of the element.

3.2. Se bioaccessibility in mushrooms

The soluble extract obtained after *in vitro* simulated gastrointestinal digestion of the selenized mushroom contained about 106 μg Se g^{-1} dw (Table 4). This means that 75% of the Se taken up by the mushroom was solubilised in conditions simulating human gastrointestinal digestion and thus was potentially bioavailable (Hur, Lim, Decker, & McClements, 2011).

For a first fractionation of Se-containing species in the gastrointestinal extract according to their molecular weight size exclusion HPLC-ICP-MS was used. Investigation of the Se molecular fractions by size exclusion HPLC-ICP-MS revealed that proteins and any other high molecular weight selenium-containing molecule were hydrolyzed to peptides and low molecular weight selenocompounds (⩽5 kDa based on column calibration, Fig. 1). A peak coeluting with a SeMet standard (retention time 36.5 min) was already visible in the size exclusion chromatogram, whereas the other major peak (retention time 34.6 min) likely corresponds to SeMet-selenoxide (SeOMet) and Se-peptides unspecifically produced by the gastrointestinal juice.

The Se not solubilized after gastrointestinal digestion might be present in form of undigestible Se-containing polysaccharides. For instance, it has been shown that part of the Se in Se-enriched mycelia of *P. ostreatus* is associated with chitin-containing structures in cell walls (Munoz et al., 2006). Formation of Se-containing polysaccharides might explain the low Se bioavailability found elsewhere for other species (Chansler et al., 1986; Mutanen, 1986). Limited bioavailability for these species might be the result of a low bioaccessibility due to a larger incorporation of Se in mushroom polysaccharides compared to *P. florida* in the conditions of the present study.

3.3. Speciation of bioaccessible Se in mushrooms

For the characterization of Se species liberated after *in vitro* enzymolysis anion exchange HPLC-ICP-MS was chosen. Fig. 2 shows that Se speciation in the gastrointestinal extract was dominated by SeMet, which represented 73% of the sum of the detected species. The addition of 0.5% v/v 2-mercaptoethanol to the extracts allowed to assess that a small percentage of SeMet (2%) was present as SeOMet (the SeMet peak increased after treatment with the reducing agent) and that some other oxidised Se-species were present. On the other hand, only 2% of the detected species was present as inorganic Se in the form of Se(IV). A number of other low molecular weigh selenocompounds generated during the gastrointestinal digestion, accounting for 25% of the chromatographed Se, remained unidentified. Overall, post column recovery (calculated as chromatographed Se *vs* total Se in gastrointestinal extracts measured by ICP-DRC-MS) was 89%.

Table 4
Concentration of Se species in mushroom gastrointestinal hydrolysates determined by anion exchange HPLC-ICP-MS (μg Se g^{-1} dw, $n = 3$).

Sample	SeMet	Se(IV)	∑other species[a]	Overall sum of species[b]	Total Se[c]
GI extract	69.2 ± 5.6	1.84 ± 0.23	23.6 ± 1.7	94.6 ± 5.9	105.8 ± 1.6

[a] Sum of Se species other than SeMet and Se(IV).
[b] Sum of SeMet, Se(IV) and ∑other species.
[c] As measured by ICP-DRC-MS.

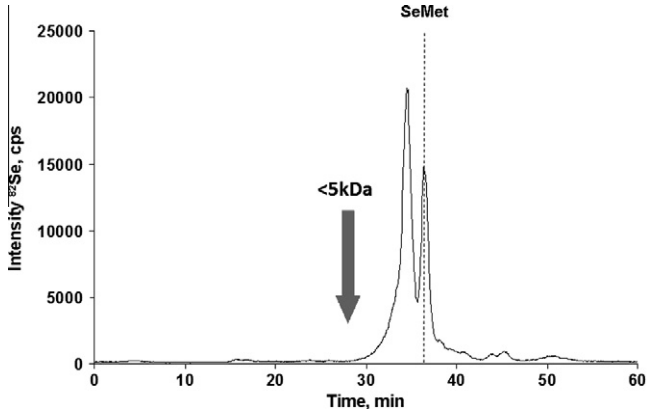

Fig. 1. Selenium molecular fractions in a mushroom gastrointestinal hydrolysate: size exclusion HPLC-ICP-MS chromatogram (see Table 2 for operating conditions).

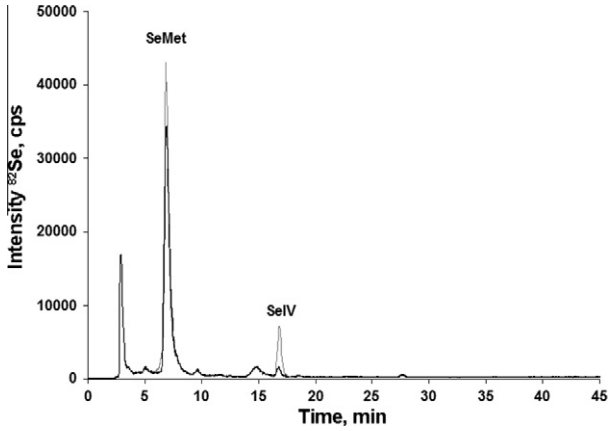

Fig. 2. Selenium species in a mushroom gastrointestinal hydrolysate: anion exchange HPLC-ICP-MS chromatogram (see Table 2 for operating conditions). Overlay of the chromatograms of the sample and the sample spiked with 250 and 50 μg Se L^{-1} of SeMet (RT ~6.5 min) and Se(IV) (RT ~17 min), respectively.

The fact that bioaccessible Se in Se-biofortified *P. florida* is in the form of organoselenium compounds, with SeMet as the major species, indicates that this species has a higher nutritional value as Se source compared to other selenized mushrooms accumulating primarily inorganic Se (Table 1). Organic forms of Se, and especially SeMet, are more bioavailable than inorganic Se and there is good evidence that increased Se status attained after supplementation with organic forms of Se is retained for a longer period after supplementation has ceased than is the case with Se(IV) or Se(VI) (Rayman et al., 2008).

The predominance of SeMet and organic Se in Se-biofortified *P. florida* is likely the result of the biosynthetic pathways involved in Se assimilation in this species. Our earlier study demonstrated that the Se-rich wheat grain used for spawn production in this work mainly contains SeMet and other organoselenium compounds (Cubadda et al., 2010) but this may not be the case of the wheat straw used as the growth substrate. Biotransformation of inorganic selenium into organoselenium compounds might be a feature of the *Pleurotus* genus, but since this is the first study to investigate Se speciation in Se-enriched oyster mushrooms further studies are needed to establish whether this is the case.

4. Conclusions

The evaluation of Se-enriched mushrooms as a possible dietary source of the element requires an understanding of the bioaccessibility and speciation of the Se accumulated from growth substrates. This study investigated *in vitro* Se bioaccessibility in *P. florida*, an edible species of oyster mushrooms, which accumulated substantial Se amounts when cultivated on naturally Se-enriched by-products from wheat production. Seventy-five percent of the Se in the mushroom fruiting body was extracted after *in vitro* simulated gastrointestinal digestion and size exclusion HPLC-ICP-MS revealed that all the Se was present in the form of low molecular weight selenocompounds. Anion exchange HPLC-ICP-MS showed that the bioaccessible Se was mainly present as SeMet, which accounted for 73% of the sum of the detected species and not less than 65% of the bioaccessible Se. Therefore, Se-biofortified *P. florida* appears to be a good bioavailable source of Se.

This study also illustrates the feasibility of producing Se-biofortified edible mushrooms employing Se-rich agricultural residues as substrates. The approach presented in this work can be used to evaluate whether Se-contaminated plant wastes may be used to produce Se-biofortified edible mushrooms based on the concentration, bioaccessibility and speciation of Se in the mushrooms.

Acknowledgements

We are indebted to Andrea Raggi for technical assistance in analytical determinations. Research grants under RGNF-UGC to Poonam Bhatia and UGC-SAP (II) to Tejo Prakash are duly acknowledged.

References

Chansler, M. W., Mutanen, M., Morris, V. C., & Levander, O. A. (1986). Nutritional bioavailability to rats of selenium in Brazil nuts and mushrooms. *Nutrition Research, 6*(12), 1419–1428.
Cheung, P. C. K. (2009). *Mushrooms as functional foods* (ed P. C. K. Cheung), John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9780470367285.ch6
CMAFP (Committee on Medical Aspects of Food Policy). (1991). *Dietary Reference Values for Food Energy and Nutrients for the UK. Report on Health and Social Subjects* (Vol. 41). The Stationery Office: London.
Combs, G. F. (2001). Selenium in global food systems. *British Journal of Nutrition, 85*(5), 517–547.
Costa-Silva, F., Marques, G., Matos, C. C., Barros, A., & Nunes, F. M. (2011). Selenium contents of Portuguese commercial and wild edible mushrooms. *Food Chemistry, 126*(1), 91–96.
Crews, H. M., Clarke, P. A., Lewis, D. J., Owen, M., Strutt, P. R., & Izquierdo, A. (1996). Investigation of selenium speciation in *in vitro* gastrointestinal extracts of cooked cod by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 11*(12), 1177–1182.
Cubadda, F., Aureli, F., Ciardullo, S., D'Amato, M., Raggi, A., Acharya, R., et al. (2010). Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *Journal of Agricultural and Food Chemistry, 58*(4), 2295–2301.
da Silva, M. C. S., Naozuka, J., da Luz, J. M. R., de Assuncao, L. S., Oliveira, P. V., Vanetti, M. C. D., et al. (2012). Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chemistry, 131*(2), 558–563.
da Silva, M. C. S., Naozuka, J., Oliveira, P. V., Vanetti, M. C. D., Bazzolli, D. M. S., Costa, N. M. B., et al. (2010). In vivo bioavailability of selenium in enriched *Pleurotus ostreatus* mushrooms. *Metallomics, 2*(2), 162–166.
Dernovics, M., Stefanka, Z., & Fodor, P. (2002). Improving selenium extraction by sequential enzymatic processes for Se-speciation of selenium-enriched *Agaricus bisporus*. *Analytical and Bioanalytical Chemistry, 372*(3), 473–480.

Dhillon, K. S., & Dhillon, S. K. (2009). Selenium concentrations of common weeds and agricultural crops grown in the seleniferous soils of northwestern India. *Science of The Total Environment, 407*(24), 6150–6156.

Diaz Huerta, V., Fernandez Sanchez, M. L., & Sanz-Medel, A. (2005). Qualitative and quantitative speciation analysis of water soluble selenium in three edible wild mushrooms species by liquid chromatography using post-column isotope dilution ICP–MS. *Analytica Chimica Acta, 538*(1–2), 99–105.

Diaz Huerta, V., Fernandez Sanchez, M. L., & Sanz-Medel, A. (2006). An attempt to differentiate HPLC-ICP-MS selenium speciation in natural and selenised Agaricus mushrooms using different species extraction procedures. *Analytical and Bioanalytical Chemistry, 384*(4), 902–907.

Diaz-Alarcon, J. P., Navarro-Alarcon, M., Lopez-Garcia de la Serrana, H., & Lopez-Martinez, M. C. (1994). Determination of selenium levels in vegetables and fruits by hydride generation atomic. *Journal of Agricultural and Food Chemistry, 42*(12), 2848–2851.

Estrada, A. E. R., Lee, H. J., Beelman, R. B., Jimenez-Gasco, M. D., & Royse, D. J. (2009). Enhancement of the antioxidants ergothioneine and selenium in *Pleurotus eryngii* var. eryngii basidiomata through cultural practices. *World Journal of Microbiology & Biotechnology, 25*(9), 1597–1607.

EUROFIR (European Food Information Resource Network). Food Composition Databases available on <http://www.eurofir.net/eurofir_knowledge/european_databases> Accessed 05.07.12.

EURRECA(EURopean Micronutrient Recommendation Alligned). Database on Dietary Recommendation available on <http://www.eurreca.org/everyone> Accessed 05.07.12.

Falandysz, J. (2008). Selenium in edible mushrooms. *Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews, 26*(3), 256–299.

FNB (Food and Nutrition Board), Institute of Medicine, the National Academies with Health Canada Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (2000). *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: National Academy Press.

Fordyce, F. M., Zhang, G. D., Green, K., & Liu, X. P. (2000). Soil, grain and water chemistry in relation to human selenium-responsive diseases in Enshi District, China. *Applied Geochemistry, 15*(1), 117–132.

Gergely, V., Kubachka, K. M., Mounicou, S., Fodor, P., & Caruso, J. A. (2006). Selenium speciation in *Agaricus bisporus* and *Lentinula edodes* mushroom proteins using multi-dimensional chromatography coupled to inductively coupled plasma mass spectrometry. *Journal of Chromatography A, 1101*(1–2), 94–102.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). *In vitro* human digestion models for food applications. *Food Chemistry, 125*(1), 1–12.

Johnson, C. C., Ge, X., Green, K. A., & Liu, X. (2000). Selenium distribution in the local environment of selected villages of the Keshan Disease belt, Zhangjiakou District, Hebei Province, People's Republic of China. *Applied Geochemistry, 15*(3), 385–401.

Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry, 113*(1), 9–16.

Kalac, P. (2010). Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000–2009. *Food Chemistry, 122*(1), 2–15.

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V., & Pizzoferrato, L. (1999). Nutrients in edible mushrooms: An inter-species comparative study. *Food Chemistry, 65*(4), 477–482.

Manzi, P., & Pizzoferrato, L. (2000). Beta-glucans in edible mushrooms. *Food Chemistry, 68*(3), 315–318.

Mattila, P., Konko, K., Eurola, M., Pihlava, J. M., Astola, J., Vahteristo, L., et al. (2001). Contents of vitamins, mineral elements, sand some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry, 49*(5), 2343–2348.

Munoz, A. H. S., Kubachka, K., Wrobel, K., Corona, J. F. G., Yathavakilla, S. K. V., Caruso, J. A., et al. (2006). Se-enriched mycelia of *Pleurotus ostreatus*: Distribution of selenium in cell walls and cell membranes/cytosol. *Journal of Agricultural and Food Chemistry, 54*(9), 3440–3444.

Mutanen, M. (1986). Bioavailability of selenium in mushrooms, *Boletus edulis*, to young women. *International Journal for Vitamin and Nutrition Research, 56*(3), 297–301.

Ogra, Y., Ishiwata, K., Encinar, J. R., Lobinski, R., & Suzuki, K. T. (2004). Speciation of selenium in selenium-enriched shiitake mushroom, *Lentinula edodes*. *Analytical and Bioanalytical Chemistry, 379*(5–6), 861–866.

Oldfield, J. E. (1999). *Selenium world atlas*. Selenium-Tellurium Development Association.

Rayman, M. P. (2000). The importance of selenium to human health. *Lancet, 356*(9225), 233–241.

Rayman, M. P. (2002). The argument for increasing selenium intake. *Proceedings of the Nutrition Society, 61*(2), 203–215.

Rayman, M. P. (2008). Food-chain selenium and human health: Emphasis on intake. *British Journal of Nutrition, 100*(2), 254–268.

Rayman, M. P., Angus, F., & Goenaga-Infante, H. (2007). Bioavailability and speciation of selenium from selenium-enriched mushrooms. *Proceedings of the Nutrition Society, 66*(Supplement OCA-B), 55A.

Rayman, M. P., Infante, H. G., & Sargent, M. (2008). Food-chain selenium and human health: Spotlight on speciation. *British Journal of Nutrition, 100*(2), 238–253.

SINU, Società Italiana di Nutrizione Umana. (1996). Livelli di Assunzione Raccomandati di Energia e Nutrienti per la Popolazione Italiana (Recommended dietary allowances for energy and nutrients for the Italian population). Available on <http://www.sinu.it/larn/mineral1.asp> Accessed 11.02.12.

Slejkovec, Z., van Elteren, J. T., Woroniecka, U. D., Kroon, K. J., Falnoga, I., & Byrne, A. R. (2000). Preliminary study on the determination of selenium compounds in some selenium-accumulating mushrooms. *Biological Trace Element Research, 75*(1–3), 139–155.

Spadoni, M., Voltaggio, M., Carcea, M., Coni, E., Raggi, A., & Cubadda, F. (2007). Bioaccessible selenium in Italian agricultural soils: Comparison of the biogeochemical approach with a regression model based on geochemical and pedoclimatic variables. *Science of the Total Environment, 376*(1–3), 160–177.

Spolar, M. R., Schaffer, E. M., Beelman, R. B., & Milner, J. A. (1999). Selenium-enriched *Agaricus bisporus* mushrooms suppress 7,12-dimethlybenz a anthracene bioactivation in mammary tissue. *Cancer Letters, 138*(1–2), 145–150.

Stefanka, Z., Ipolyi, I., Dernovics, M., & Fodor, P. (2001). Comparison of sample preparation methods based on proteolytic enzymatic processes for Se-speciation of edible mushroom (*Agaricus bisporus*) samples. *Talanta, 55*(3), 437–447.

Vetter, J., Hajdu, C., Gyorfi, J., & Maszlaver, P. (2005). Mineral composition of the cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. *Acta Alimentaria, 34*(4), 441–451.

Wang, X. D. C., Jiang, H., Chen, Z., Shi, H., & Li, J. (2005). The effect of the Se to the fruiting-body of *Pleurotus ostreatus* and nutrition composition. *Food Science, 26*(8), 91–95.

Wilburn, R. T., Vonderheide, A. P., Soman, R. S., & Caruso, J. A. (2004). Speciation of selenium in the mushroom *Boletus edulis* by high-performance liquid chromatography coupled to inductively coupled plasma-mass spectrometry with a collision cell. *Applied Spectroscopy, 58*(10), 1251–1255.

Wuilloud, R. G., Kannamkumarath, S. S., & Caruso, J. A. (2004). Multielemental speciation analysis of fungi porcini (*Boletus edulis*) mushroom by size exclusion liquid chromatography with sequential on-line UV-ICP-MS detection. *Journal Agricultural Food Chemistry, 52*(5), 1315–1322.

Yoshida, M., Sugihara, S., Inoue, Y., Chihara, Y., Kondo, M., Miyamoto, S., et al. (2005). Composition of chemical species of selenium contained in selenium-enriched shiitake mushroom and vegetables determined by high performance liquid chromatography with inductively coupled plasma mass spectrometry. *Journal of Nutritional Science and Vitaminology, 51*(3), 194–199.

Zhao, L., Zhao, G. H., Zhao, Z. D., Chen, P., Tong, J. Y., & Hu, X. S. (2004). Selenium distribution in a se-enriched mushroom species of the genus Ganoderma. *Journal of Agricultural and Food Chemistry, 52*(12), 3954–3959.

J Nutr Sci Vitaminol, **59**, 69–72, 2013

Note

Selenium Uptake by Edible Oyster Mushrooms (*Pleurotus* sp.) from Selenium-Hyperaccumulated Wheat Straw

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(Received July 30, 2012)

Summary In an effort to produce selenium (Se)-fortifying edible mushrooms, five species of oyster mushroom (*Pleurotus* sp.), were cultivated on Se-rich wheat straw collected from a seleniferous belt of Punjab, India. Total selenium was analyzed in the selenium hyperaccumulated wheat straw and the fruiting bodies. Significantly high levels ($p<0.0001$) of Se uptake were observed in fruiting bodies of all mushrooms grown on Se-rich wheat straw. To the best of our knowledge, accumulation and quantification of selenium in mushrooms has hitherto not been reported with substrates naturally enriched with selenium. The results demonstrate the potential of selenium-rich agricultural residues as substrates for production of Se-enriched mushrooms and the ability of different species of oyster mushrooms to absorb and fortify selenium. The study envisages potential use of selenium-rich agricultural residues towards cultivation of Se-enriched mushrooms for application in selenium supplementation or neutraceutical preparations.

Key Words selenium, uptake, wheat straw, *Pleurotus*

Historically, the image of selenium (Se) has changed dramatically over the last century. Initially, selenium was widely considered to be a toxic agent in mammals. It was first recognized to be an essential trace element in 1957 and has been shown to be active in glutathione peroxidase (GSH-Px) in red blood cells. Since its discovery about half a century ago, selenium has been a subject of intensive research. Selenium, an essential micronutrient with antioxidant properties, has received considerable attention for potential roles in cancer prevention for both human beings and animals (*1*). One of the first and best known examples of the physiological importance of Se is the family of selenium-dependent glutathione peroxidases, that protect membrane lipids and phospholipids from oxidative stress by catalyzing reduction of hydrogen peroxide, lipid and phospholipid hydroperoxide (*2*). The element also (a) seems active at supra-nutritional levels of dietary intake, mostly in the field of cancer prevention (*3–6*); (b) may possess pharmacological implications as an adjuvant treatment of some cancers (*7*); and (c) facilitates enhancement of intrinsic defenses against ROS (*8*). In humans, it is well recognized that selenium plays a crucial role in various physiological processes and its altered level has a direct impact on health leading to various disorders. The World Health Organization report advises a Se intake of 40 μg/d as the average intake level needed to ensure meeting normative requirements of healthy adults.

With increasing demand for nutritional food across the world, enrichment of staple diets with essential micronutrients such as Se has been gaining importance. Selenium deficiency is linked with various conditions such as increased cancer and infection risk, male infertility, decrease in immune and thyroid function, and several neurologic conditions, including Alzheimer's and Parkinson's disease (*5*) and increase in urinary ketone bodies in starved rats (*9*). The majority of the population across Scandinavia and other European countries is at risk of Se deficiency, because of which various supplementation strategies are being attempted, including naturally fortified dietary intake. Selenium deficiency can be significantly tackled by its supplementation through dietary sources (*10*). Different food sources of plant and animal origin are known to be potential dietary sources of selenium. The main dietary constituents providing selenium in the diet are bread, meat, fish, eggs, and milk/dairy products (*11*). Among the crop plants, wheat, maize, pulses and certain vegetables crops such as *Allium sativum*, broccoli, garlic and mushrooms are known to be good sources of selenium.

Mushrooms are interesting food items for the formulation of a healthy diet as they are devoid of high calories and fat but are rich in proteins, minerals and dietary fibre (*12*, *13*). They have been extensively studied for their nutritional and medicinal properties. Mushrooms have known antioxidant properties provided by different compounds such as phenolics, ergothioneine and selenium (Se) (*14–17*). Natural mushrooms such as *Boletus edulis* for example, may have concentrations of up to 17 mg Se/kg dw, while wild *Agaricus* sp. may contain 2.7 mg Se/kg dw and *P. cornucopiae* and *Grifola frondosa* may have less than 0.5 mg Se/kg dw (*16*, *18*). Selenium content in mushrooms is observably species

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specific (*19*). The main bioavailable species of selenium in *Agaricus bisporus* (*20*) and *Lentinula edodes* (*21*) cultivated on selenium-supplemented substrates was found to be selenomethioneine under conditions where exogenous supplementation of selenium was carried out using inorganic Se species, reports indicate an increase in the Se content in mycelia as well as fruiting bodies of mushrooms (*15*, *16*, *22*). Werner and Beelman (*15*) also demonstrated that Se accumulated in *Agaricus bisporus* when the substrate was supplemented with aqueous solutions of selenite at different concentrations and that the Se uptake by *A. bisporus* was linearly related to concentration in the compost.

In the present study, five different species of genus *Pleurotus* (Oyster mushroom) comprising commercially important edible as well as medicinal important species were considered. These species were cultivated on Se-rich wheat straw collected post-harvest from a seleniferous region of Punjab, India. *Pleurotus* mushrooms have high nutritional value and can be a good source of protein, carbohydrates, vitamins, calcium and iron (*23*). Reported health-related properties of these genera include reduction of cholesterol levels, antitumor, antiviral, antibacterial and immunomodulating activity (*24*).

The present study demonstrates the use selenium rich agricultural residues to cultivate different species of oyster mushrooms (*Pleurotus* sp.) and suggests the use of these residues as a prospective raw material for generating Se-fortified mushrooms.

Materials and Methods

Analytical grades of 2,3-diaminonaphthalene (DAN, Hi-Media) and cyclohexane (SD-Fine, India) were used in the study. Nitric (HNO_3), hydrochloric (HCl) and perchloric ($HClO_4$) acids were procured from Merck, India.

The strains of *Pleurotus djamor*, *P. sajorcaju*, *P. ostreatus*, *P. citrinopileatus* and *P. fossulatus* were obtained from NRCM, Solan (Himachal Pradesh, India). All strains were cultured on potato dextrose agar (PDA) medium and stored at 4°C for further use. The selenium-rich agricultural residues of wheat harvested from a seleniferous belt (32°46′ N, 74°32′ E) of Punjab, India, were used as substrates for mushroom cultivation. The test mushrooms were cultivated on agricultural residues collected from the test site, following the method outlined by Punjab Agricultural University (*25*) with minor modifications. Spawn was prepared on wheat grains and was used for seeding of Se-rich substrate. The wheat straw was treated with formalin for the purpose of disinfection. The straw was soaked overnight in water containing formalin (1.5%). The disinfected straw was allowed to air dry before the inoculation of the substrate with the fungal spawn. After inoculation, the substrate was layered in polypropylene bags and kept in a cultivation chamber set at 25±4°C. The moisture was maintained by spraying water over the culture bags twice a day. A similar procedure was followed to cultivate control mushrooms, with non-Se wheat straw as the substrate. On maturity (about 30 d after spawning), fruiting bodies were collected, by cutting the base of the stipe with sterile surgical blade, weighed and dried at 40°C in an oven for near complete dehydration. The dry samples were macerated using an agate mortar and pestle.

Selenium content in powdered samples was analysed using a fluorescence spectrometric method (*26*). In brief, a 100 mg portion of the sample was weighed accurately in an acid-washed kjeldahl flask containing 5 mL concentrated nitric acid. After incubation for 30 min at room temperature, 2 mL of perchloric acid (72%) was added and the sample was digested. The mixture was then allowed to cool to room temperature. To facilitate the reduction of selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}), 2 mL of 1.0 N HCl was added and the flask was placed in the water bath (100°C) for 15 min. The final volume of the digest was adjusted to 25 mL with 0.1 N HCl. To 1.0 mL of the diluted digest, 200 μL of 1 : 1 formic acid and 200 μL of stabilizing solution [0.04 M Na_2EDTA containing 10% hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$)] were added. The final pH of the digest was adjusted to 1.8 with NH_4OH and placed in a water bath at 50°C for 10 min. 0.5 mL DAN (0.1% in 0.1 M HCl) was added to the reaction mixture, shaken thoroughly (20 s) and kept in the water bath at 50°C for 30 min. After cooling to room temperature, 2 mL of cyclohexane were added, and the contents were vigorously mixed, and allowed to separate. The separated cyclohexane layer was washed with 5 mL 0.1 N HCl. The emission spectrum of piazselenol complex formed during the reaction was measured using a fluorescence spectrometer (Perkin Elmer LS-45) at excitation and emission wavelengths of 320 and 520 nm respectively. Se quantification in each sample was carried out by the relative method using emission spectrum of NIST certified Selenium ICP standard solution (SRM-1349).

Results and Discussion

The study presents the observations on uptake and accumulation of Se among different species of *Pleurotus* cultivated using wheat straw naturally enriched with Se as the substrate. Different species of *Pleurotus* genus were examined for their potential to take up and accumulate Se. Selenium concentration in wheat straw used as growth substrate and in fruiting bodies of different mushrooms is shown in Table 1. Mushrooms cultivated on naturally Se-enriched substrates did not differ from controls in terms of yield (expressed as fresh mushroom weight per bag) or production length (number of days elapsed from inoculation to harvest).

The selenium concentration in unenriched substrate was found to be higher (1.9±0.8 μg Se/g dw) than in the earlier reports which lay in the range of 0.09–0.19 μg Se/g dw in different substrates (*27*, *28*) and the resulting concentrations in fruiting bodies were in the range of 2.9–5.2 μg Se/g dw, which is almost comparable to the range (0.12–3.4 μg Se/g dw) of selenium conentartion in wild and edible species of *Pleurotus* (*29–31*). The fruiting bodies (first flush) of all species of *Pleurotus* harvested from Se-rich wheat straw containing a total Se concentration of 24.0±0.2 μg Se/g dw, were noted to

Table 1. Total selenium levels in substrate and different species of Selenium-enriched/control mushrooms.

Sample	Total selenium (μg/g dw)					
	Straw	Mushroom				
		P. djamor	*P. ostreatus*	*P. sajorcaju*	*P. fossulatus*	*P. citrinopileatus*
Se	24.0±0.2	145.4±2.9	44.3±2.3	43.5±2.1	37.2±0.6	26.1±2.8
Control (Non-Se)	1.9±0.8	5.0±0.5	3.4±0.1	5.2±1.0	3.5±0.5	2.9±0.14

Values are presented as mean±SD (n=3).

accumulate significantly higher ($p<0.0001$) selenium as compared to control mushrooms grown on control wheat straw (1.9±0.8 μg Se/g dw).

Among the five strains of *Pleurotus*, *P. djamor* was found to accumulate significantly high levels of Se (expressed as μg Se/g dw) as compared to control samples i.e. 145.4±2.9 vs 5.0±0.46, followed by *P. ostreatus*, *P. sajorcaju*, *P. fossulatus* and *P. citrinopileatus* (Table 1). The extent of accumulation was higher than the selenium concentration reported in the case of *Pleurotus eryngii* (4.6 and 9.3 μg Se/g dw) cultivated on substrates supplemented with 5.0 and 10.0 μg Se/g dw of sodium selenite respectively (*32*), Similar results have recently been reported by da Silva et al. (*27*), where selenium accumulation in *P. ostreatus* was found to be higher (57.6 μg Se/g dw) than our observations (44.3 μg Se/g dw) with the same species when cultivated on Se-enriched coffee husks supplemented with 3.2 mg/kg of sodium selenite. Total selenium content in enriched *L. edodes* and *Ganoderma lucidium* was found to be 46 μg Se/g and 72 μg Se/g respectively (*33*, *34*), which lies within the range observed in the present study. The observations clearly demonstrated that the tolerance and uptake of selenium by various species of mushrooms are dependent upon the variety of mushroom species as well as the form of selenium supplementation.

Although investigations have been carried on Se uptake and activity of Se-enriched mushrooms, by other researchers, on substrates supplemented with inorganic selenium compounds (*30*, *35*) such as selenite, to the best of our knowledge, there is no information on Se levels in mushroom grown on substrates naturally enriched with selenium. As absorption of Se in the body depends on its bioavailability and speciation, with the organic species being effectively bioavailable (*4*), it is important to examine the extent of uptake of Se from substrates that have significantly high levels of organo-selenium complexes (*36*). Substrates, such as wheat straw, naturally enriched with selenium have organo-selenium species that are envisaged to have potential physiological and pharmacological benefits (*36*). Further, preliminary findings on mushrooms cultivated on substrates naturally enriched with Se were the significantly high levels of SeMet when compared to controls (data not shown). In this context, it is important to note that Se-rich mushrooms have been effective in retarding chemically induced tumors when fed to mice (*37*, *38*). Keeping this in view, mushrooms fortified/enriched in Se from natural sources might possess significant levels of bioaccessible forms of selenium, thus facilitating enhanced bioavailability and bioaccessibility of selenium required at therapeutic or neutraceutical levels, through diet.

The present study, thus, proposes the use of Se-rich agricultural residues as substrates for mushroom cultivation for human and livestock supplementation.

Acknowledgments

Research grants under UGC-SAP (II) to Dr. Tejo Prakash and RGNF-UGC to Poonam Bhatia are duly acknowledged.

REFERENCES

1) Berry MJ. 2005. Insights into the hierarchy of selenium incorporation. *Nature* **37**: 1162–1163.
2) Brigelius-Flohe R. 2006. Glutathione peroxidases and redox-regulated transcription factors. *Biol Chem* **387**: 1329–1335.
3) Finley JW, Davis C, Feng Y. 2000. Selenium from high-selenium broccoli is protective against colon cancer in rats. *J Nutr* **130**: 2384–2389.
4) Finley W. 2006. Bioavailability of selenium from foods. *Nutr Rev* **64**: 146–151.
5) Rayman MP. 2000. The importance of selenium to human health. *Lancet* **356**: 233–241.
6) Rayman MP. 2005. Selenium in cancer prevention: A review of the evidence and mechanism of action. *Proc Nutr Soc* **64**: 527–542.
7) Himeno S, Imura H. 2000. New aspects of physiological and pharmacological roles of selenium. *J Health Sci* **46**: 393–398.
8) Zhou X, Ji WJ, Zhu Y, Li H, Huang TG, Li YM. 2007. Enhancement of endogenous defenses against ROS by supra-nutritional level of selenium is more safe and effective than antioxidant supplementation in reducing hypertensive target organ damage. *Med Hypotheses* **68**: 952–956.
9) Yoshida M. 1991. Effect of selenium deficiency on urinary ketone body excretion in starved rats. *J Nutr Sci Vitaminol* **37**: 425–434.
10) Ximenez-Embun P, Alonso I, Madrid-Albarran Y, Camara C. 2004. Establishment of selenium uptake and species distribution in lupin, Indian mustard and sunflower plants. *J Agric Food Chem* **52**: 832–838.
11) Fairweather-Tait JS, Bao Y, Broadley RM, Collings R, Ford D, Hesbeth DJ, Hurst R. 2011. Selenium in human health and disease. *Antioxid Redox Signal* **14**: 1337–1383.

12) Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. 1999. Nutrients in edible mushrooms: an inter-species comparative study. *Food Chem* **65**: 477–482.
13) Manzi P, Pizzaferrato L. 2000. β glucans in edible mushrooms. *Food Chem* **68**: 315–318.
14) Mau JL, Lin HC, Song SF. 2002. Antioxidant properties of several specialty mushrooms. *Food Res Int* **35**: 519–526.
15) Werner AW, Beelman RB. 2002. Growing high selenium edible and medicinal mushroom (*Agaricus bisporus* (J. Lge) Imbach) as ingredients for functional food or dietary supplements. *Int J Med Mush* **4**: 167–171.
16) Beelman RB, Royse DJ. 2006. Selenium enrichment of *Pleurotus cornucopiae* (Paulet) and *Grifola frondosa* (Dicks.: Fr.) S.F. gray mushrooms. *Int J Med Mush* **8**: 1–8.
17) Dubost NJ, Beelman RB, Royse DJ. 2007. Influence of selected cultural factors and postharvest storage on ergothioneine content of common button mushroom *Agaricus bisporus* (J. Lge) Imbach (Agaricomycetideae). *Int J Med Mush* **9**: 163–176.
18) Piepponen S, Liukkonen-Lilja H, Kuusi T. 1983. The selenium content of edible mushrooms in Finland. *Z Lebensm Unters Forsch* **177**: 257–260.
19) Stijve T. 1977. Selenium content of mushrooms. *Z Lebensm Unters Forsch* **164**: 201–203.
20) Diaz Huerta V, Sanchez MLF, Sanz-Medel A. 2006. An attempt to differentiate HPLC ICP-MS selenium speciation in natural and selenised *Agaricus* mushroom using different species extraction procedures. *Anal Bioanal Chem* **384**: 902–907.
21) Yoshida M, Sugihara S, Inoue Y, Chihara Y, Kondô M, Miyamoto S, Sukcharoen B. 2005. Composition of chemical species of selenium contained in selenium-enriched shiitake mushroom and vegetables determined by high performance liquid chromatography with inductively coupled plasma mass spectrometry. *J Nutr Sci Vitaminol* **51**: 194–199.
22) Stajic M, Sikorski J, Wasser SP, Nevo E. 2005. Genetic similarity and taxonomic relationships within the genus *Pleurotus* (higher Basidiomycetes) determined by RAPD analysis. *Mycotaxon* **93**: 247–255.
23) Schmidt P, Welscher FS, Nascimento J, Junior FMV. 2003. Pretreatment effects on fiber degradation of brachiaria hay by *Pleurotus ostreatus* fungus. *Revista brasiliena de Zootecnia* **32**: 1866–1871.
24) Sarangi I, Ghosh D, Bhutia SK, Mallick SK, Maiti TK. 2006. Anti-tumour immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. *Int Immunopharmacol* **6**: 1287–1297.
25) Khanna PK. 2003. Mushroom growing. *In*: Mushroom Cultivation (Singh AP, eds), p 1–60. Punjab Agricultural University Press, Ludhiana.
26) Levesque M, Vendette ED. 1970. Selenium determination in soil and plant materials. *Can J Soil Sci* **51**: 85–93.
27) da Silva MCS, Naozuka J, da Luz JMR, de Assuncao LS, Oliveira PV, Vanetti MCD, Bazzolli DMS, Kasuya MCM. 2012. Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chem* **131**: 558–563.
28) Lee CY, Park JE, Kim BB, Kim SM, Ro HS. 2009. Determination of mineral components in the cultivation substrates of edible mushrooms and their uptake into fruiting bodies. *Mycobiology* **37**: 109–113.
29) Andersen ALSE, Lange M, Bech K. 1982. Trace elements in edible mushrooms. *Stat Levnedsmiddelinst Publ* **68**: 29.
30) Costa-Silva F, Marques G, Matos CC, Barros AIRNA, Nunes FM. 2011. Selenium contents of Portuguese commercial and wild edible mushrooms. *Food Chem* **126**: 91–96.
31) Vetter J, Hajdu C, Gyorfi J, Maszlaver P. 2005. Mineral composition of the cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. *Acta Alimentaria* **34**: 441–451.
32) Estrada AER, Lee HJ, Beelman RB, Jimenez-Gasco MD, Royse DJ. 2009. Enhancement of the antioxidants ergothioneine and selenium in *Pleurotus eryngii* var. *eryngii* basidiomata through cultural practices. *World J Microbiol Biotechnol* **25**: 1597–1607.
33) Gergely V, Kubachka KM, Mounicou S, Fodor P, Caruso JA. 2006. Selenium speciation in *Agaricus bisporus* and *Lentinula edodes* mushroom proteins using multidimensional chromatography coupled to inductively coupled plasma mass spectrometry. *J Chromatogr A* **1101**: 94–102.
34) Falandysz J. 2008. Selenium in edible mushrooms. *J Environ Sci Health C* **26**: 256–299.
35) Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Slate DK, Chalker CJ, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL Jr, Park H, Sander BB Jr, Smith CL, Taylor JR. 1996. Effect of Se supplementation for cancer prevention in patient with carcinoma of the skin. A randomized controlled trial. *J Am Med Assoc* **276**: 1957–1963.
36) Cubadda F, Aureli F, Ciardullo S, D'Amato M, Raggi A, Acharya R, Reddy AVR, Tejo Prakash N. 2010. Changes in selenium speciation as associated with increase in tissue concentrations of selenium in wheat grain. *J Agric Food Chem* **58**: 2295–2301.
37) Spolar MR, Schaffer EM, Beelman RB, Milner JA. 1999. Selenium-enriched *Agaricus bisporus* mushrooms suppress 7,12-dimethylbenz(a)anthracene bioactivation in mammary tissue. *Cancer Lett* **138**: 145–150.
38) Che C, Yingmei Z, Yanchao L, Huiyuan C, Yaming Z. 2007. In vivo and in vitro anti-tumour response of selenium-protein polysaccharide extracted from rich selenium *Agaricus blazei*. *Food Agric Immunol* **18**: 139–149.

Acta Alimentaria, Vol. 43 (2), pp. 280–287 (2014)
DOI: 10.1556/AAlim.43.2014.2.12

SELENIUM UPTAKE AND ASSOCIATED ANTI-OXIDANT PROPERTIES IN *PLEUROTUS FOSSULATUS* CULTIVATED ON WHEAT STRAW FROM SELENIFEROUS FIELDS

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(Received: 17 November 2012; accepted: 12 January 2013)

The present study was carried out to examine the antioxidant activity in oyster mushroom cultivated on selenium (Se) rich substrate. *Pleurotus fossulatus* was cultivated on Se-rich wheat straw collected from the seleniferous belt of Punjab (India) and its potential to accumulate Se from substrate was examined. Using different assay systems the modulations in the anti-oxidant profile of Se enriched mushroom was studied in comparison to the mushrooms cultivated on normal straw. The oyster mushrooms were observed to potentially mobilize Se from Se-rich substrates to fruiting bodies, resulting in significantly high uptake (37.2±0.6 μg g^{-1}) as compared to control (3.57±0.53 μg g^{-1}). The antioxidant activity, as determined by various assays, such as reducing power, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging, and metal chelating activity, was higher in the experimental mushrooms when compared to control. The results obtained demonstrate that Se-fortified mushrooms through cultivation on straw containing organic forms of Se can be considered as natural and effective dietary supplements of organic Se for humans. The present study proposes the use of Se-rich agricultural residues as substrates for mushroom cultivation for human and livestock supplementation.

Keywords: antioxidants, selenium, *Pleurotus* sp., fortification

Selenium (Se) is established as an essential trace mineral of fundamental importance to human health (Papp et al., 2007). Many of its physiologic roles are directly attributed to its presence within selenoproteins, such as cellular GPx (GPx1) and phospholipid hydroperoxide GPx (PHGPx; GPx4), iodothyronine 5'-deiodinases (IDI), and selenoprotein W (Burk & Levander, 1999). However, in several regions of the world the content of Se in diet has been estimated insufficient to facilitate the optimal activity of protective selenoenzymes.

Edible flora that accumulate elements, such as Se, may be used as a natural source of mineral supplement for both animals and human beings, especially in areas that are mineral deficient, through the process of biofortification. In this context, due to their high volume of production, seasonal independence, their safety, and wide acceptance by consumers all over the globe, edible mushrooms have potential to facilitate the bioavailability of such supplements (De Assuncao et al., 2012). Mushrooms have known antioxidant properties provided by different compounds, such as phenolics, ergothioneine, and Se (Beelman & Royse, 2006). The content of Se in mushrooms is species specific (Stijve, 1977).

Mushrooms belonging to genus *Pleurotus* have important medicinal properties, such as anti-tumour and immunostimulatory activity. The products derived from the fruiting bodies of this genus can promote biological responses favouring cancer treatment in humans and have been used as antitumourigenic drugs (Sarangi et al., 2006). These edible mushrooms are known to be Se accumulators. However, the amount of Se is dependent on the species, the

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stage of maturity, the amount in soil, and the substrates used for the growth of cultivated species (Kalac, 2009). Mushroom species grow and yield on a spectrum of agricultural residues and by-products, such as paddy, wheat, sugarcane bagasse, water hyacinth, rubber wood dust, and tree leaves (Khanna, 2003). The agri-wastes generated in seleniferous region of Punjab contain significant levels of Se accumulated in plant parts, as reported in leaves and straw of wheat, rice, and cereals (Dhillon & Dhillon, 2003), and therefore have potential use as substrates for cultivation of Se enriched mushrooms, leading to their appropriate utilization. Extensive research has been carried out on Se uptake by edible mushrooms cultivated on substrates supplemented exogenously with inorganic Se, but to the best of our knowledge no study has been carried out on mushrooms cultivated on substrates hyper-accumulated with Se through natural processes. Keeping this in view, the present study was aimed at exploring the cultivation of *Pleurotus fossulatus* on Se-rich wheat straw and determining its antioxidant profile as induced by Se enrichment.

1. Materials and methods

Standards tert-butylhydroquinone (TBHQ) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, USA). 2-tert-Butyl-4-methoxyphenol (BHA), gallic acid (GA), quercetin and 2,3-diaminonaphthalene (DAN) were purchased from Hi-Media (India). Methanol and cyclohexane were obtained from SDFine (Mumbai, India). Nitric (HNO_3), hydrochloric (HCl), and perchloric ($HClO_4$) acids were procured from Merck (Bengaluru, India). The rest of the reagents were obtained from Loba Chemie, Mumbai, India.

The strain of *P. fossulatus* was procured from National Research Centre of Mushroom (NRCM), Solan (India). This fungus was cultured on potato dextrose agar (PDA) medium and was stored at 4 °C until use. The Se-rich agricultural residues of wheat crop collected from the village of Jainpur (31°13' N, 76°21' E, Nawanshahr-Hoshiarpur region, Punjab, India) were used as substrates for mushroom cultivation. The mushrooms were cultivated on agricultural residues collected from this site, following the method outlined by Punjab Agricultural University, India (Khanna, 2003) with minor modifications. Similar conditions were followed to grow control mushrooms, with non-Se wheat straw as the substrate. At maturation (22–25 days after inoculation) fruiting bodies were collected and dried at 40 °C for near complete dehydration.

The Se content of powdered samples (from first flush) was analysed using florescence spectrometry (Levesque & Vendette, 1971). The emission spectrum of piazselenol complex formed during the reaction was measured using fluorescence spectrometer (Perkin Elmer LS-45) at excitation and emission wavelength of 360 and 520 nm, respectively. Se quantification in each sample was carried out by the relative method using emission spectrum of NIST certified Se-ICP standard solution (SRM-1349).

Prior to use, the fruiting bodies were milled until a fine powder was obtained. The total protein content in the mushroom was determined by the method of Lowry and co-workers (1951). One gram of the samples in case of estimations of total phenols and total antioxidants and 100 mg in case of other assays were subjected to stirring with 10 ml of 20% and 90% methanol for 2 h, respectively, using ultrasonicator bath, and filtered through Whatman 1 paper. The resultant methanolic extracts (ME) were stored at 4 °C until use. Total phenolic content of both extracts (20% ME and 90% ME) was analysed using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) using gallic acid as standard.

Thiobarbituric acid reactive substances (TBARS) were assayed fluorometrically (Perkin-Elmer LS45) according to the method of Minotti and Aust (1987). Calibration curves were made using malondialdehyde (MDA; Sigma, St. Louis, USA) in the range of 0.5–5.0 μM. The total antioxidant activity of the methanolic extract (20 and 90% methanol) of *P. fossulatus* was measured using UV-visible spectrometer (Hitachi U-2900) according to the method (phospho-molybdenum assay) outlined by Imran and co-workers (2011), wherein gallic acid (GA) (0.01–0.1 mg ml^{-1}) was used as standard.

The scavenging activity of 20% ME and 90% ME from mushroom on DPPH radicals was measured spectrophotometrically following the method of Chu and co-workers (2000) using quercetin (0.05–0.50 mg ml^{-1}) as standard. The scavenging activity (%SA) of DPPH radicals was calculated using the equation

$$\%SA = \frac{A_{control} - A_{sample}}{A_{control}} \times 100.$$

The chelating activity of the various concentrations of extracts (20% ME and 90% ME) for ferrous ions was measured spectrophotometrically following the ferrozine method (Dinis et al. 1994). The metal chelating activity of the mushroom extracts was calculated as:

$$\%\ \text{chelating activity} = \frac{A_{negative} - A_{sample}}{A_{negative}} \times 100,$$

where A is absorbance. EDTA was used as positive control, while absence of the mushroom extract was the negative control.

All estimations were carried out in triplicates except for total protein content, which was done in duplicates. The comparison between Se and non-Se samples was drawn with Student's *t* test using Graphpad Prism Ver.5.0.

2. Results and discussion

2.1. Protein content in fruiting bodies

Total protein content in methanolic extracts of Se enriched mushrooms (307±4.5 mg g^{-1} d.w.) was found to be significantly higher ($P<0.01$) than of control mushrooms (282±2.4 mg g^{-1} d.w.) (Table 1). The total protein contents (205–246 mg g^{-1} d.w.) of different species of *Pleurotus* grown on non-enriched substrates (Alam et al., 2008) and Se enriched *Lentinus edodes* (213.9±1.09 mg g^{-1} d.w.) (Turlo et al., 2010) were found to be lower than our findings.

The chemical composition of edible mushrooms determines their nutritive value. The Se in mushrooms is dominantly found as selenocysteine, selenomethionine, and methylselenocysteine in addition to inorganic Se (Cremades et al., 2012). Since mushrooms have relatively high protein levels, and can accumulate large amounts of Se, it is reasonable to expect that this element could be significantly incorporated in proteins.

Table 1. Total selenium (n=3) in substrates and fruiting bodies of *P. fossulatus* and total protein content (n=2) in fruiting bodies (n=3)

Sample	Se (μg g^{-1} d.w.)		Total proteins (mg g^{-1} d.w.)
	Wheat straw	Fruiting bodies	Fruiting bodies
Se	24.0±0.2	37.2±0.6	307±4.5
Non-Se	1.90±0.8 ***	3.57±0.53 ***	282±2.4 **

**: P<0.01; *** P<0.001

2.2. Se in substrate and fruiting bodies

The Se contents in straw collected from Se-rich and control (non-Se) sites and in fruiting bodies of Se-rich mushrooms cultivated on the said substrates are presented in Table 1. The fruiting bodies harvested from Se-rich straw containing a total Se concentration of 24.0±0.2 μg g^{-1} were noted to accumulate significantly higher (P<0.001) Se up to 37.2±0.6 μg g^{-1} as compared to control/non-Se mushrooms (3.57±0.53 μg g^{-1}) cultivated on non-Se straw (1.9±0.8 μg g^{-1}). The extent of accumulation was notably higher than the Se concentrations of 4.6 and 9.3 μg g^{-1} reported for *P. eryngii* cultivated on substrates supplemented with 5.0 and 10.0 mg kg^{-1} of sodium selenite, respectively (Estrada et al., 2009), but lower than those reported in *P. ostreatus* (57.6 μg g^{-1}) cultivated on substrate supplemented with 3.2 mg kg^{-1} of sodium selenite (Da Silva et al., 2012).

Many investigations have been carried out with growing Se-enriched mushrooms on substrate supplemented with Se, wherein dominantly inorganic forms of Se compounds were used for the supplementation (Costa-Silva et al., 2011). However, as there is no report on the mobilization of Se by mushrooms, such as *P. fossulatus,* from substrates naturally enriched with Se, use of such substrates can facilitate the availability of bioaccessible forms of Se during growth of mushrooms.

2.3. Se induced transformations in antioxidant properties of mushrooms

The influence of Se hyperaccumulation in *P. fossulatus* was examined on the antioxidant properties of mushrooms using various assay systems. Among the various antioxidant compounds, polyphenols have gained importance due to their large array of biological actions that include free radical scavenging, metal chelation, enzyme modulation activities, and inhibition of LDL oxidation, among others (Rodrigo & Bosco, 2006). In addition, Se is well known for its association with antioxidant mechanisms in humans and animals. The total phenolic content, expressed as mg GA/g d.w. of mushrooms, is shown in Table 2. The amount of phenolic compounds in the methanol (20%) extracts from the Se-enriched mushroom (11.15±0.37 mg GA/g d.w.) was significantly higher (P<0.01) than the control (9.32±0.18 mg GA/g d.w). Similarly, the total phenol content in 90% methanolic extract from Se-enriched mushroom (7.10 ±0.17 mg GA/g d.w.) was also found to be significantly higher (P<0.05) than the control (6.45±0.10 mg GA/g d.w.). However, mushrooms in 20% ME showed higher phenol content than in 90% ME. The results obtained were higher than the total phenol content (4.0±0.32 mg g^{-1}) reported in case of Se-enriched fruiting of *Agaricus bisporus* (Cremades et al., 2012) but comparable to the phenol content (14.3 mg g^{-1} in water extracts; 7.4 mg g^{-1} in methanolic extracts) reported in case of *P. sajorcaju* (Puttaraju et al., 2006)

grown on non enriched substrates. Increase in the phenol content in Se-enriched mushrooms may be due to the influence of Se in promoting the synthesis and activity of antioxidant metabolites, a feature that has been observed in the present study, as well. Various researchers have earlier defined role of Se in inducing antioxidant capacity by facilitating increase in the amounts of tocopherol and phenolic compounds in plants (Xu et al., 2003).

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation phase of lipid oxidation. Antioxidant activity in terms of free radical scavenging activity of non enriched mushroom extracts is well reported (Puttaraju et al., 2006). Compared to those results, the present study showed that normal (non-Se) fruiting bodies of *P. fossulatus* have higher activity in terms of radical scavenging. On the other hand, Se-rich extracts (20% methanol) showed significantly higher ($P<0.001$) scavenging activity (33.6±1.0%) than non-selenated (21.5±0.4%) extracts (Table 2).

Lipid peroxidation is one of the main manifestations of oxidative damage. Lipid peroxides act on the cellular components, leading to both structural and functional damage of the bimolecular as well as the cellular structure. Mushrooms are considered to generate phenolic antioxidants to inhibit lipid peroxidation (Yen & Chen, 1995). The present observations showed that the MDA content was significantly lower in Se rich extracts (51.5±3.0 nM MDA/g d.w.) ($P<0.001$) compared to control (105.9±2.8 nM MDA/g d.w.) (Table 2), which suggests the inhibition of lipid peroxidation.

Metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalyzing transition metal in the lipid peroxidation process (Dodig & Cepelak, 2004). The chelating effect of Se rich mushrooms from both extractions (20% ME and 90% ME) was found to be significantly higher ($P<0.05$) than non-enriched mushrooms. The 20% ME from Se-enriched and control samples showed the chelating ability of 56.5±2.07% and 44.7±3.5%, respectively, likewise in 90% ME from Se-enriched and control mushrooms showed chelating ability of 53.6±3.3% and 45.8±0.48%, respectively (Table 2).

The results suggested that moderate ferrous-ion chelating ability showed by Se-enriched mushroom extracts could be beneficial to health. Iron can stimulate lipid peroxidation by the Fenton reaction and can also accelerate peroxidation by decomposing lipid hydroperoxide into peroxide and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain of lipid peroxidation (Halliwell, 1999). The high metal chelating activity of Se-enriched mushrooms is assumed to be due to specific metal-Se interactions that greatly affect antioxidant activity based on the type of metal and the specific features of the Se containing biological moieties.

The total anti-oxidant content of the methanol extracts of mushrooms were measured spectrophotometrically with the phosphomolybdenum assay. The total antioxidant content of the methanol extracts of Se-enriched mushrooms was significantly higher than the control (Table 2). Se-enriched mushroom as 90% ME showed higher ($P<0.001$) antioxidant content (41.9±1.15 mg BHT/g d.w.) than non-enriched extracts (34.7±0.65 mg BHT/g d.w.). Similar results ($P<0.05$) were obtained in case of extracts prepared in 20% ME, although lesser amounts were measured than in 90% ME.

The results in the present study revealed that methanolic extracts of Se-enriched mushrooms act as free radical scavengers, indicating the potential use of Se-rich mushrooms for Se supplementation through diet.

Table 2. DPPH scavenging potential, metal chelating efficacy, total antioxidant activity, and lipid peroxidation inhibition capacity of methanolic extracts from Se enriched and control *P. fossulatus* (n=3)

	DPPH scavenging potential (%)		Total phenols (mg GA/g)		Metal chelating activity (%)		Total antioxidant activity (mg GA/g)		Lipid peroxidation (nM MDA/g)
	90% ME	20% ME	90% ME	20% ME	90% ME	20% ME	90% ME	20% ME	
Se-rich	40.60±2.87	33.63±1.01	7.10±0.17	11.1±0.37	53.60±3.33	56.52±2.07	41.93±1.15	32.8±0.3	51.5±2.8
Control	36.03±0.74	21.59±0.40	6.45±0.10	9.32±0.18	45.86±0.48	44.74±3.59	34.76±0.65	31.0±1.0	105.9±3.0
	ns	***	*	**	**	**	***	*	***

*P<0.05; ** P<0.01; *** P<0.001; ns: non-significant

3. Conclusion

The present study demonstrates the use of Se-rich agricultural residues as substrates for cultivation of Se-enriched *P. fossulatus* (oyster mushrooms), which hitherto has been the only one reported with sole exogenous Se supplementation. *Pleurotus fossulatus* indicated notable Se accumulation and corresponding antioxidant activities on cultivation using substrates naturally enriched with selenium. Se-enriched mushrooms with enhanced antioxidant content can therefore serve as effective dietary supplements or neutraceuticals. Thus, the present study proposes the use of Se-rich agricultural residues as substrates for mushroom cultivation for human and livestock Se supplementation.

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Research grants under UGC-SAP (II) to Dr. N.Tejo Prakash and RGNF-UGC to Poonam Bhatia are duly acknowledged.

References

Alam, N., Amir, R., Khan, A., Ara, I., Shim, M.J., Lee, M.W. & Lee, T.S. (2008): Nutritional analysis of cultivated mushrooms in Bangladesh- *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocbye indica*. *Mycobiology, 36*, 228–232.

Beelman, R.B. & Royse, D.J. (2006): Selenium enrichment of *Pleurotus cornucopiae* (Paulet) Rouant and *Grifola frondosa* (Dicks: Fr.) S.F. Gray mushrooms. *Int. J. Med. Mushrooms.*, *8*, 1–8.

Burk, R.F. & Levander, O.A. (1999): Selenium -in: Shils, M.E., Olson, J.A., Shike, M. & Ross, A.C. (Eds) *Modern nutrition in health and disease*. Lippincott Williams & Wilkins, Baltimore, MD., pp. 265–276.

Chu, Y.H., Chang, C.L. & Hsu, H.F. (2000): Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Fd Agric., 80*, 561–566.

Costa-Silva, F., Marques, G., Matos, C.C., Barros, I.R.N.A. & Nunes, F.M. (2011): Selenium contents of Portuguese commercial and wild edible mushrooms. *Fd Chem.*, *26*, 91–96.

Cremades, O., Herrero, M.M.D., Aguilar, P.C., Gil, J.F.G., Fontiveros, E., Morgado, B.R., Parrado, J. & Bautista, J. (2012): Preparation and characterization of selenium enriched mushroom aqueous enzymatic extracts (MAEE) obtained from the white button mushroom (*Agaricus bisporus*). *Fd Chem.*, *133*, 1538–1543.

Da Silva, M.C.S., Naozuka, J., Oliviera, P.V., Vancetti, M.C.D., Bazzolli, D.M.S. & Kasuya, M.C.M. (2012): Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Fd Chem.*, *131*, 558–563.

De Assuncao, L.S., De Luz, J.M.R., Da Silva, M.C.S., Vieira, P.A.F., Bazzolli, D.M.S., Vanetti, M.C.D. & Kasuya, M.C.M. (2012): Enrichment of mushrooms: An interesting strategy for acquisition of lithium. *Fd Chem.,* 134*,* 1123–1127.

Dhillon, K.S. & Dhillon, S.K. (2003): Quality of underground water and its contribution towards selenium enrichment of the soil-plant system for a seleniferous region of northwest India. *J. Hydrol., 272*, 120–130.

Dinis, T.C.P., Madeira, V.M.C. & Almeida, L.M. (1994): Action of phenolic derivatives (acetoaminophen, salicylate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys., 315*, 161–169.

Dodig, S. & Cepelak, I. (2004): The facts and controversies about selenium. *Acta Pharmacol.*, *54*, 261–276.

Estrada, A.E.R., Lee, H.J., Beelman, R.B., Jimenez-Gasco, M.D. & Royse, D.J. (2009): Enhancement of the antioxidants ergothioneine and selenium in *Pleurotus eryngii* var. *eryngii* basidiomata through cultural practices. *World J. Microbiol. Biotechnol., 25*, 1597–1607.

Halliwell, B. (1999): Antioxidant defense mechanisms: from the beginning to the end. *Free Radical Res.*, *31*, 261–272.

Imran, M.M., Raja, M.M., Basith, J.A. & Asarudeen, A. (2011): Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *Int. Fd Res. J., 18*, 574–577.

Kalac, P. (2009): Chemical composition and nutritional value of European species of wild growing mushrooms. A review. *Fd Chem.*, *113*, 9–16.

Khanna, P.K. (2003): Mushroom growing –in: Singh, A.P (Ed.) *Mushroom cultivation*. Punjab Agricultural University Press, Ludhiana, pp. 1–60.

Levesque, M. & Vendette, E.D. (1971): Selenium determination in soil and plants. *Can. J. Soil Sci.*, *51*, 85–93.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, *193*, 265–275.

Minotti, G. & Aust, S.D. (1987): The requirement for iron(III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. *J. Biol. Chem.*, *262*, 1098–1104.

Papp, L.V., LU, J., Holmgren, A. & Khanna, K. (2007): From selenium to selenoproteins: synthesis, identity and their role in human health, *Antioxid. Redox Sign., 7*, 776–806.

Puttaraju, N.G., Venkateshaiah, S.U., Dharmesh, S.M., Urs, S.M.N. & Somasumandaram, R. (2006): Antioxidant activity of indigenous edible mushrooms. *J. Agric. Fd Chem., 54*, 9764–9772.

Rodrigo, R. & Bosco, C. (2006): Oxidative stress and protective effects of polyphenols: comparative studies in human and rodent kidney: A review. *Comp. Biochem. Physiol. C.*, *142*, 317–327.

Sarangi, I., Ghosh, D., Bhutia, S.K., Mallick, S.K. & Maiti, T.K. (2006): Anti-tumour immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. *Int. Immunopharmacol.*, *6*, 1287–1297.

Singleton, V.L. & Rossi, J.A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic., 16*, 144–158.

Stijve, T. (1977): Selenium content of mushrooms. *Z. Lebensm. Unters. Forsch*, *164*, 201–203.

Turlo, J., Gutkwosaka, B. & Herold, F. (2010): Effect of selenium enrichment on antioxidant activities and chemical composition of *Lentinula edodes* (Berks) Pegl. Mycelial extracts. *Fd Chem. Toxicol., 48*, 1085–1091.

Xu, J., Yang, L., Chen, L., Hu, Y. & Hu, Q. (2003): Effect of selenium on increasing the antioxidant activity of tea leaves harvested during the early spring tea producing season. *J. Agric. Fd Chem., 51*, 1081–1084.

Yen, G.C. & Chen, H.Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Fd Chem.*, *43*, 27–32.