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Mineral Element Enrichment of Mushrooms for the Production of More Effective Functional Foods

V.O. Oyetayo

Department of Microbiology, Federal University of Technology, Akure, Nigeria

ABSTRACT

In the last three decades, human awareness of the promotion of good health through dietary intervention had increased. Hence, there has been advocacy for the promotion and consumption of health-enhancing foods referred to as functional foods. Myconutrients and chemicals obtained mainly from higher fungi have also received attention as sources of bioactive compounds that can serve as functional foods. Moreover, the importance of mineral elements in our diet for the maintenance of human health cannot be over-emphasized. These elements are responsible for a lot of chemical and electrical processes that enhance the stable homeostatic physiological state in the body and their absence normally results in deficiency diseases. These processes are highly specified and specialized and the human body can only function properly if the accurate balance of minerals and other elements is continually being supplied to look after our system. Most staple foods consumed by humans are lacking in one essential mineral element or the other. It is therefore expedient to find a way to remedy this. Edible and medicinal mushrooms that can easily absorb minerals and can bio-accumulate them as functional organic compounds during growth can therefore be used as a vehicle to supply these mineral elements that are not in adequate amounts in our diet. This will certainly be a definite strategy to solve the problem of mineral malnutrition. Some of these essential elements are selenium, iron, zinc, calcium and so on. This paper, therefore, highlights the potential of mushrooms as a hydra-headed vehicle for the supply of mycoactive compounds and essential minerals in our diets as a means of promoting human health.

KEYWORDS

Health, enhancing, functional foods, strategy, mineral elements, enrichment

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INTRODUCTION

One of the key concerns of man is the maintenance of good health. It is popularly said that health is wealth. Food, which is one of the necessities of life outside shelter and cloth, is very essential in the maintenance of good health. It is needed to achieve a stable homeostatic physiological status in humans to prevent diseases and ailments. A diseased state is often described as a deviation from the normal physiological state and food with the required healthy constituents has been found to help in maintaining this stable physiological state. Food has been considered the major factor in maintaining well-being and

health from the beginning of human history¹. Hippocrates, who was considered the father of Western medicine, stated as far back as 400 BC, "Let thy food be thy medicine and thy medicine be thy food". In essence, Hippocrates recognized the relationship between food and good health. Despite the submission of Hippocrates, foods were only recognized as being nutritious based on their content of essential nutrients such as fats, protein, carbohydrates, minerals and vitamins.

In the last three decades, growing scientific evidence has demonstrated that other bioactive food constituents, often referred to as phytochemicals and zoochemical, may provide a health benefit beyond basic nutrition and help prevent chronic diseases like cardiovascular disease, diabetes, cancer and osteoporosis among others. These foods that can enhance good health are referred to as functional foods in Europe while, America adopted the term Nutraceuticals. The word functional foods and nutraceuticals are often used interchangeably. However, Cencic and Chingwaru² differentiated between the two terms by stating that functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, minerals etc, needed for its healthy survival while nutraceuticals on the other hand help in the prevention and treatment of diseases apart from preventing anaemia.

Functional food or medicinal food is any fresh or processed food claimed to have a health-promoting and/or disease-preventing property beyond the basic nutritional function of supplying nutrients³. Functional food must be food, not a drug. Beneficial effects should be obtained by consuming normal amounts of functional food within the 'normal' diet. The ultimate goal of the scientific community and food industry is to develop functional foods for improving life quality⁴. Functional foods are therefore used to enhance certain physiological functions and to prevent or even cure diseases⁵. The term 'functional food' was coined in Japan in the 1980s and refers to processed foods containing ingredients that aid specific body functions, in addition to being nutritious⁶. Currently, there is no universally accepted term for functional foods. A variety of terms have appeared worldwide such as nutraceuticals, medifoods, vitafoods, custom foods, designer foods and the more traditional dietary supplements and fortified foods. The Japanese were the first to observe that food could have a role beyond gastronomic pleasure and energy and nutrient supply to the human organism. Following this observation, Japan is the country where most functional foods are on the market and the first country to legislate these products in the FOSHU (Foods of Specified Health Use) legislation. However, functional foods are generally considered as those foods which are intended to be consumed as part of the normal diet and that contain biologically active components which offer the potential of enhanced health or reduced risk of disease⁷. Examples of functional foods include foods that contain specific minerals, vitamins, fatty acids or dietary fibre, foods with added biologically active substances such as phytochemicals or other antioxidants and probiotics that have live benefits. According to this definition, unmodified whole foods such as fruits and vegetables represent the simplest form of functional food. Plant foods such as broccoli, carrots or tomatoes are rich in such physiologically active components as sulforaphane, beta carotene and lycopene, respectively which made them be physiologically active when consumed.

According to Hassler⁶, the present interest and developments in functional foods stem from:

- Increased scientific understanding of the importance of food constituents and properties for health
- C Opportunities for the food industry to develop, on this basis, foods with added value that the consumer is willing to pay for and
- New possibilities to increase consumers' health and well-being and to help combat current diet-related diseases

Functional foods in the last three decades in the western world has be a new revolution and there is a rapid growth of functional food industries while in the orient, functional foods have been a part of the culture for centuries. In Traditional Chinese Medicine, foods that have medicinal effects have been documented since at least 1000 BC. From ancient times, the Chinese have believed that foods have both preventive and therapeutic effects and are an integral part of health, a view that is now being increasingly recognized around the world⁸.

Mushrooms are known to be pharmacologically active; hence they are important in food and medicine. Edible mushrooms are known to be a source of healthy foods. They contain high-quality digestible protein that varied between (10-40%), carbohydrates (3-21%) and dietary fibre (3-35%) on a dry weight basis depending on the species⁹. Pharmacologically, the activities of mushrooms have been linked to the bioactive compounds present in them. The health-promoting properties of edible and medicinal mushrooms have been associated with the presence of numerous bioactive compounds present in them. Major bioactive compounds found in mushrooms include polysaccharides, proteins, terpenes, phenolic compounds and unsaturated fatty acids and many other substances of different origins^{10,11}. The fruit bodies and the mycelium of some medicinal mushrooms are known to contain compounds with wide-ranging medicinal properties¹². The use of mushrooms as a healthy food has been enhanced based on their rich proteins and minerals and poor calories and fat¹³. Health-promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol-lowering, immunostimulatory and many others had been associated with the consumption of mushroom $14-16$.

Mushrooms can also absorb mineral elements and can bioaccumulate them as functional organic compounds during growth. Mineral nutrients are indispensable to the maintenance of life. These elements are very important for cell functions at biological, chemical and molecular levels 17 . Human nutritional requirements demand at least 23 mineral elements. Some of these essential elements are selenium, iron, zinc, calcium and so on. This article, therefore, focused on the potential of using mushrooms as a hydra-headed functional food for the supply of myconutraceuticals and essential mineral elements in our diets as a means of promoting human health.

Mineral elements and their importance in health promotion: The daily intake of several minerals is a necessity for the continued basic functioning of the human body. Hence, human needs both macronutrients and micronutrients for the maintenance of good health; however, most people are aware of the importance of macronutrients such as carbohydrates, fats, proteins and vitamins in health promotion while no cognizance is taken of micronutrients. The body mass of the man is made up of 98% of nine nonmetallic elements. The absence of these metals in adequate amounts can affect the metabolic process which can lead to disease. Etiopathogenesis of many nutritional disorders has also been linked with interactions among these trace elements though they constitute only 0.02% of the body weight, they play significant roles as active co-enzymes or trace bioactive substances^{17,18}. Volumes of scientific data from physiologic investigations have revealed that inadequate consumption of these micronutrients (minerals and trace elements) can affect the optimal absorption and utilization of other nutrients to work effectively in the body. Mineral elements are needed in adequate amounts to regulate important metabolic and structural functions in the human body. Mineral elements which constitute a very small portion of the body weight are very important in all activities of the body. They are essential participants in every metabolic process carried on by the body. It has been observed that humans required more than 22 mineral elements which can be supplied by an appropriate diet¹⁹. A balanced diet must of necessity contain these required mineral elements in adequate amounts.

Mineral elements are categorized as micro- or macro-minerals based on the amount needed in the human diet to maintain good nutrition. Microelements or trace elements are required in amounts as low as a few milligrams or less per day. Other microelements are required in lesser amounts as low as micrograms per day. These are referred to as ultra-trace elements. Common examples of microelements are iron, zinc, copper, manganese, selenium, iodine, chromium and molybdenum. On the other hand, macroelements are mineral elements required in hundreds of milligrams to several grams per day. Some examples of macroelements are calcium, phosphorus, magnesium, potassium and so on. The importance of some of these mineral elements is individually stated below.

Copper (Cu): Copper is a micronutrient needed at milligrams less per day. The daily requirement is about 2-5 mg out of which about 50% is absorbed from the Gastrointestinal Tract (GIT)¹⁷. Copper (Cu) is an essential trace element in both humans and animals. It is needed only in trace amounts, the human body contains approximately 100 mg Cu. It is also involved in a myriad of biological processes such as antioxidant defense, neuropeptide synthesis and immune function $20,21$. Copper plays a significant role in human metabolism because it allows many critical enzymes to function properly²². The solubility of copper is more pronounced in acidic conditions where copper ions are incorporated into the food chain in the cupric or cuprous form¹⁷. In the human body, copper accumulates in the liver, brain and kidney. Over 90% of plasma copper is associated with ceruloplasmin and 60% of Red Blood Cell (RBC) is bound to superoxide dismutase. Copper is required for the normal growth and health of the human body. It plays a role in the formation of connective tissue and the normal functioning of muscles and the immune and nervous systems¹⁷. Copper and iron are required for the formation of red blood cells. Copper also influences the functioning of the heart and arteries, helps prevent bone defects such as osteoporosis and osteoarthritis and promotes healthy connective tissues²³. Copper is involved in cell metabolism and is a part of various enzymes such as tyrosinase, uricase and cytochrome oxidase, which are mainly concerned with oxidation reactions 17 .

Bost et al.²³ reported that copper level in foodstuff is affected by factors such as soil Cu concentration, slurry/manure spreading and use of Cu compounds as microbiocides on crops. Moreover, copper content in cereals, fruit and vegetables and, in meat and animal products is also affected by copper emissions from smelting and casting industries^{24,25}. Food groups such as offal and nuts are rich in copper while to a lesser extent cereals and fruit are also good sources of Cu. However, milk and dairy products have been reported to contain low amounts of Cu^{26} .

Copper deficiency in our diet can lead to anaemia, growth retardation, defective keratinization and pigmentation of hair, hypothermia, mental retardation, changes in the skeletal system and degenerative changes in aortic elastin. On the other hand, when copper is in excess in our diet or through other sources, it can produce nausea, vomiting, diarrhoea, profuse sweating and renal dysfunction¹⁷. The following had been identified as the symptoms of copper deficiency: hypochromic anaemia, neutropenia, hypopigmentation of hair and skin, abnormal bone formation with skeletal fragility and osteoporosis, joint pain, lowered immunity, vascular abnormalities and uncrimped or steely hair²⁷.

Zinc (Zn): Zinc is a trace mineral element that played important role in gene expression, cell development and replication²⁸. It is therefore essential to all forms of life. Zinc is needed for growth and must be in sufficient amounts in tissues such as the immune and gastrointestinal tract that are involved in rapid cellular differentiation²⁹. The average body content of zinc in an adult is 2-3 g and the average daily requirement is 15-20 mg/day¹⁷. The level of zinc intracellularly is about 99% while the rest is in the plasma. It is required for the catalytic activity of approximately 100 enzymes 17 .

Zinc is also required for a proper sense of taste and smell. The body does not possess a specialized zinc storage system; hence a daily intake is required for the body to maintain a steady state³⁰. Zinc is found in abundance and the absorbable form in the organs and flesh of mammals, fowl, fish and crustaceans since these foods do not contain phytate that can chelate it. The content of zinc in fruits and vegetables is not high except for spinach which contains a fairly high zinc density²⁹. Zinc deficiency is characterized by growth retardation, loss of appetite and impaired immune function^{31,32}. Summarily, zinc is essential for

body growth, maturation and development as well as tissue repair and resistance to disease. It is important for children and the aged. Deficiency of zinc in the diet of children can result in reduced growth, while in adults it can lead to reduced infection and delayed wound healing in people of all ages. The body needs 15.0 milligrams of zinc per day.

Magnesium (Mg): Magnesium is a macromineral element that is very essential to the proper functioning of the body. It is a vital nutrient and is the active mineral in at least 300 known enzymes in the human body33. The Recommended Daily Intake (RDI) for magnesium for adult males is 400 mg/day with slightly lower for children and women. Magnesium is required to help the body do the following: Produce energy, protein synthesis, muscle and nerve function control, blood glucose control and blood pressure regulation.

Magnesium plays a very important role in the electrical functions of the heart to keep a regular beat³³. Other functions of magnesium in the body include the maintenance of healthy blood pressure³⁴, control of blood glucose^{35,36}. Magnesium deficiency can cause a wide variety of features including hypocalcaemia, hypokalaemia and cardiac and neurological manifestations. A chronic low magnesium state has been associated with several chronic diseases including diabetes, hypertension, coronary heart disease and osteoporosis.

Iron (Fe): Iron is an essential metal element for life, though it is one of the most abundant metals on earth; however, it is not readily available for use³⁷. Dietary iron is necessary for the production of new red blood cells. It is a component of haemoglobin, which transports oxygen from the lungs to other tissues in the body and it's also found in myoglobin, which is necessary for the storage and diffusion of oxygen in muscle cells. Iron is important in the synthesis of many important cellular components such as Adenine Triphosphate (ATP), transport of oxygen, Deoxyribonucleic Acid (DNA) and electron transport³⁸. Anaemia has been associated with the deficiency of iron³⁹. Moreover, Zhang *et al*.⁴⁰ associated the increasing level of malnutrition with the diminishing level of iron in our foods.

Selenium (Se): Selenium is a component of over 25 different proteins called selenoproteins. Selenoproteins are important in a wide array of physiological processes. Moreover, it is important in the synthesis of glutathione, critical detoxification and free radical neutralizing enzyme⁴¹. Dietary selenium has been recognized as an antioxidant and the deficiency of this element has been associated with numerous chronic degenerative diseases, including multiple types of cancer, cardiomyopathy and endemic osteoarthropathy⁴². Its optimal intake could potentially prevent various types of cancer and diseases like diabetes, age-related immunosuppression and even problems related to fertility⁴³.

Manganese (Mn): Manganese is an essential nutrient in many ways. Its key role is in the activation of enzymes that are needed for the digestion and utilization of foods and nutrients. It also plays a role in reproduction and bone growth. It is sometimes called the 'brain' mineral as it is important to mental function44. Manganese deficiency is very rare and hard to determine. However, many people may not be getting the optimal levels needed for health. The most common cause of low manganese is a poor dietary intake, either due to a diet lacking in manganese food sources or because of intestinal tract disorders that hinder the absorption of nutrients from food. Table 1 and 2 contain some macro and micro-mineral elements, sources and functions.

Mineral element enrichment of mushrooms: The enrichment of mushrooms with mineral elements can be achieved by growing the mushroom with substrate already spiced with the mineral element of interest. In the case of enrichment with Iron (Fe), the mushroom will be exposed to iron at different concentrations. The cultivation involves cutting and transferring one agar disc (8.0 mm) of an actively growing mushroom to the centre of an already prepared pure culture medium (PDA, Merck, Darmstadt, Germany) containing

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0, 50.0 or 100.0 mg L $^{-1}$ of iron in form of FeSO₄ \cdot 7 H₂O at pH 5.5. This will be followed by the incubation of the plates at 25°C for 7 days. The growth of the mushroom formed after 7 days will be assessed by measuring the colony's diameter in two directions that are perpendicular to each other⁵². The biomass (dry mass) will be determined by emptying the entire contents of the Petri dish (mycelium + culture media) into a bottle with distilled water and heating in a water bath (5-10 min) to dissolve the culture medium. Then, the solution will be filtered and the mycelium will be dried in an oven at 60°C until a constant weight will be reached $53,54$.

The substrate can be injected with the mineral element of interest⁵⁵. Briefly, for selenium, the substrates (corn cobs) will be sundried and broken into smaller pieces using mortar and pestle. It will be further pulverized into a fine powder using a mill machine. The substrate (corn cobs) will be moistened with water to a concentration of 60%. One thousand grams (1000 g) of the substrate will be packed into polypropylene bags and sealed with paper with the aid of polyvinyl rings. The bags will then be autoclaved for 2 hrs at 121°C. The substrates will be allowed to cool down and then inoculated with 30 g of mushroom spawn. Sodium selenite (50 mg kg^{-1}) will be injected into the substrate. A control with no sodium selenite will also be prepared to serve as a control. The bags will then be kept in the spawn room at 75% relative humidity until the formation of primordial. The bags will then be uncapped and transferred to the fruiting room to allow the normal development of the fruitbodies. The total Se content can then be determined using an inductively coupled plasma mass spectrometry (Agilent ICP-MS 7900, Agilent Technologies, Santa Clara, CA, USA).

Some experimental results: One of the strategies adopted to combat dietary or mineral inadequacies is food bio-fortification with minerals. Bio-fortification of foods with mineral elements is feasible, relatively cheap, efficient and safer than other diet supplementation approaches in preventing nutritional deficiencies⁵⁶. Bio-enrichment of foods is a conspicuous tactic to solve the occurrence of ailments associated with nutrient deficiencies. Supplementation of the mineral into cultivated *Pleurotus* spp., can be embraced to treat symptoms of macro- and micro-element deficiencies. Mushrooms have great potential for uptake and accumulate various elements in their fruiting bodies. Mushrooms are bio-fortified with certain elements; lithium⁵⁷, iron⁵⁸, selenium⁵⁵ and zinc⁵⁹ have the potential of being used as nutritional therapy. Based on the functions of Zinc, Selenium and iron, they are indispensable elements that need to be integrated into food crops to enhance the health of the end consumers.

Oyetayo *et al.*⁶⁰, the total phenol in *Pleurotus* spp not grown on substrate fortified with iron and zinc were 10.7 mg GAE/g of the extract while the *Pleurotus* spp fortified with Fe, Zn and Zn+Fe had 13.3, 15.8 and 16.7 mg GAE/g of extract, respectively. An increase was also observed in the flavonoids content of *Pleurotus* spp fortified with Fe, Zn and Zn+Fe with flavonoid values of 7.0, 8.2 and 8.4 mg QE/g of extract compared with the unfortified *Pleurotus* spp., with flavonoid content of 5.8 mg QE/g. Gąsecka *et al*. 61 obtained higher phenolic compounds in fortified mushrooms, *P. ostreatus* and *P. eryngii* concurrently enriched with Se and Zn with the value of 13.38 mg g $^{-1}$ of extract and 10.86 mg g $^{-1}$ of extract, which was higher than control. Medicinal mushrooms synthesize a great number of phenolic compounds like polyphenols, hydroxybenzoic acids, flavonoids, tannins, hydroxycinnamic acids, stilbenes, 4-hydroxybenzoic, ferulic, p-coumaric, protocatechuic, t-cinnamic, vanillic acids, cinnamic acid and lignans with other secondary metabolites; like lectins, lactones, terpenoids, alkaloids^{61,62}. Flavonoids are very important bioactive constituents with a variety of biological potentials like a free radical scavengers, metal chelators and various physiological activities.

Extracts from mushrooms fortified with Zn have the highest scavenging activity (96.3%) against DPPH. However, the OH⁻ scavenging activity of extract from mushrooms fortified with Zn (96.3%) and the control, BHT (97.1%) were not significantly different. Extract from non-fortified mushroom, mushroom fortified with Zn and BHT has a similar scavenging activity of 95.0, 96.8 and 97.1% against NO radical.

Extracts from mushrooms bio-enriched with Se and Se+Zn displayed significant antioxidant activities by improving Reactive Oxygen Species (ROS) and inhibiting lipid peroxidation⁶³. Supplementation of mushroom growth substrate with selenite and Zn sulfate significantly increased DPPH scavenging activity in fruiting bodies. Bio-fortified mushrooms have greater antioxidative properties and could enhance oxidative stress in humans. Bhatia *et al*. 64 had earlier reported that extracts of the Se-enriched *P. sajor-caju* and *Volvariella volacea* mushroom spp., exhibited higher reducing and scavenging activities than the non-enriched *P. sajor-caju*. Moreover, the study conducted by Poniedzialek *et al*. 63 reported that biofortification of *P*. *ostreatus* and *P*. *eryngii* with selenium significantly improved their antioxidant and reducing activities, indicating the potential applicability of such bio-fortified ingredients as a functional food. In another study on the nutraceutical property of *Pleurotus* enriched with selenium, it was observed that selenium-enriched *Pleurotus* spp., showed significant antioxidant and antimicrobial properties^{55,65}.

Safety assessment: There is a need for a safety assessment of mineral element enriched mushrooms. This will help to determine if the permissible level(s) of these mineral elements have not been exceeded. The safety and nutritional effect of the mushroom fortified with mineral elements still need to go animal bioassay. This may involve the evaluation of the enriched mushrooms in rats fed dietary inclusions of the mineral element fortified mushroom. Relevant haematological, nutritional and safety parameters such as liver function tests could be monitored in the fed rats to determine their level of safety.

Future perspective: The importance of mineral nutrients in the maintenance of life cannot be overemphasized. Though, the required daily intake of these mineral elements is small compared particularly with nutrients such as carbohydrates and lipids, they are, however, indispensable components that must be present in a healthy diet. The absence of some of these mineral elements in our food had often been associated with one deficiency disease or the other. It is therefore expedient that these mineral elements are adequately supplied through our food. One way of doing this is through enrichment of our foods with these elements.

CONCLUSION

Mushrooms can absorb mineral elements and can bio-accumulate them as functional organic compounds during growth. Mushrooms can therefore be used as a vehicle to supply minerals that are not in adequate amounts in our diet. Some of these essential elements that can be bio-accumulated by mushrooms are selenium, iron, zinc, copper and so on. In one of our studies, *Pleurotus* spp., enriched with selenium showed significant antioxidant and antimicrobial properties. Therefore, selenium-enriched *Pleurotus* spp., could serve as a rich source of natural antioxidants and antimicrobial food for the enhancement of the body against oxidative damage and pathogenic invasion. Fortification of *Pleurotus* spp., with essential metals should therefore be encouraged as this can improve its application as health-promoting foods.

SIGNIFICANCE STATEMENT

The present review revealed the potential of mushrooms as a vehicle that can be used to introduce essential elements into our body through the consumption of mushrooms fortified with essential mineral elements. Mineral element enrichment of mushrooms is therefore a definite strategy to solve the problem of mineral element deficiency which is common as a result of eating foods that lack these essential nutrients. Furthermore, the consumption of mineral-enriched mushrooms possesses a double advantage of supplying useful mycochemicals and essential minerals that can help in promoting stable physiological homeostasis in man.

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