

Incorporation of a ^{75}Se Label into *Agaricus bisporus*

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Introduction

Since the 1950s selenium has been recognized as an essential animal nutrient and there is increasing evidence that it is also required for proper human nutrition. A major function of selenium in humans is the protection of cell membranes. In the form of selenocysteine, selenium functions at the active site of the enzyme glutathione peroxidase (7). This enzyme catalyzes the two electron reduction of hydroperoxides using glutathione as the reducing agent. Hydroperoxides can attack the double bonds of unsaturated fatty acids of phospholipids in cell membranes. The resulting lipid peroxides can react further causing cellular free radical damage. Glutathione and glutathione peroxidase reduce the lipid peroxides preventing the cleavage of carbon-carbon bonds and disruption of cell membranes (8).

Presently there is no recommended dietary allowance for selenium but there is a safe and adequate intake suggested by the Food and Nutrition Board of the National Academy of Sciences. A selenium concentration of 0.1 μg selenium per gram of diet is adequate for optimal growth and reproduction in all mammalian species studied. A range of 50 to 200 μg per day is suggested as adequate and safe for adults (5). Selenium intakes in this range are not difficult to obtain when a varied diet is consumed. Human disease states that are associated with selenium deficiency and respond to selenium therapy have been identified. Keshans disease, a fatal cardiomyopathy, and Kaschin-Beck disease, a joint and muscle degenerative disease, occur among children and young women in the Keshan region of China where soil levels of selenium are low and intakes of the mineral are nearly zero (2,6).

Plants grown where soil conditions are favorable for the uptake of selenium will accumulate the mineral but little is known about the chemical form of selenium in plants. The chemical form of selenium influences the biological availability of the mineral. Bioavailability is assessed in animal studies by comparing various food sources of selenium to selenite in the ability to restore blood and liver levels of selenium and glutathione peroxidase activities after a depletion diet.

Selenium is found to be associated with protecin in foods. Some major food sources of selenium are seafoods, organ meats and muscle meats. Cereals are also an important source of dietary selenium in the United States and Canada (3). Mushrooms are of interest because they have been reported to have higher selenium contents than most foods in the fruit and vegetable categories (9). Little is known about the relative nutritional availability of selenium from these foods.

Foods differ widely in their ability to regenerate glutathione peroxidase activity and raise selenium levels in blood and liver. This is suspected to be related to differing chemical forms of selenium in the foods. Selenium in wheat has been found to be 83.0% as effective as selenite in restoring glutathione peroxidase activity (3), whereas selenium from mushrooms was only 4 to 28.0% as effective as selenite (1).

Selenium in bacteria, plants, and animals has been found in proteins as analogs of the sulfur containing amino acids, cysteine and methionine. About half of the selenium in wheat is in the form of selenomethionine (10). Preliminary studies in our laboratory indicate that a high percentage of selenium in soybeans is associated with high molecular weight storage proteins. Currently, research is underway to determine the exact chemical form of selenium in soybeans. Researchers in Finland have shown selenium in mushrooms

to be associated with protein and free amino acids (Piepponen, 1984 personal communication).

The purpose of the study reported here was to determine if *Agaricus bisporus* mushrooms could be grown successfully in a growth chamber and to see if a radioisotope of selenium could be incorporated into the mushrooms. This was a preliminary study to one in which the chemical form of selenium in *Agaricus bisporus* will be determined.

Materials and Methods

Three basic materials are necessary for mushroom growth. These include, 1) compost, 2) spawn, and 3) casing soil to cover the beds after the spawn has grown through the compost (4).

Compost was obtained from a commercial source (Cansco, Inc., Howe, Indiana). *Agaricus bisporus* spawn was obtained from Mushroom Science Labs, Avondale, PA. Forty grams of spawn were mixed with enough wetted compost to form a six to eight inch layer in the bottom of a five gallon bucket. The compost was tamped so that it was tight and the buckets were covered with black plastic. Growth chamber temperature was maintained at 24°C while the spawn was colonizing the compost. The compost was watered as necessary with a light stream of deionized water.

When more than half of the top of the compost was covered by mycelium, a one inch layer of autoclaved peat casing, pH 7.2, was spread over the compost. At this time two different treatments were initiated. The first treatment was the introduction of 1 μ Ci of ⁷⁵Se-sodium selenate in deionized water directly into the compost before it was cased. The second treatment was the introduction of the 1 μ Ci dose of ⁷⁵Se-sodium selenate into the casing material before it was spread over the compost. Thereafter treatment of both groups was identical. The black plastic cover was removed and a small fan placed in the growth chamber to lower carbon dioxide levels near the beds. The casing was watered with a fine mist of deionized water to prevent packing of the peat. The temperature of the growth chamber was maintained at 24°C for four days following casing. Thereafter the temperature was decreased slowly so that at the time of harvest air temperature was 16°C.

The first pinheads appeared sixteen days after casing. The first mushrooms were harvested ten days later. Mushrooms were weighed and frozen until harvest was completed.

Results and Discussion

Accumulation of ⁷⁵Se by mushrooms in the two treatment groups is shown in Table I. The compost-labeled mushrooms weighed 419g when cleaned and homogenized and incorporated 6.1% of the ⁷⁵Se dose. The casing-labeled mushrooms weighed 211g when cleaned and homogenized and incorporated 2.3% of the ⁷⁵Se dose. Therefore, incorporation of a ⁷⁵Se label into *Agaricus bisporus* was most efficient when introduced into the compost.

The mushrooms from the compost-labeled group had a smooth surface while those from the casing-labeled group had a cracked or scaly surface. This cracking is thought

TABLE 1. Accumulation of ⁷⁵Se by *Agaricus bisporus* mushrooms

Treatment	Yield(g)	% Uptake of ⁷⁵ Se dose
Compost-labeled	419	6.1
Casing-labeled	211	2.3

to be due to a draft created by the fan which was positioned closer to the casing labeled bed.

The lower efficiency of incorporation of the ^{75}Se label into the casing-labeled group may have resulted from poor transport of the ^{75}Se -sodium selenate to the compost where nutrients are absorbed. After casing, the beds were watered with a fine mist and there may not have been enough water flow to carry the sodium selenate to the compost. The more rapid drying of the casing soil in this treatment due to the positioning of the fan may also have contributed to the water flow problem and decreased yield. Mushrooms are over 90% water and during fruit body formation large amounts of moisture are drawn from the compost.

Results of this study show that a ^{75}Se label can be incorporated into *Agaricus bisporus*. The significance of this is that a radiolabel will aid in the purification of selenium-containing compounds in the mushrooms and in the identification of the chemical form of selenium in the purified compounds. Also, ^{75}Se labeled mushrooms can be incorporated into the diets of laboratory animals to study the bioavailability of selenium from this food source. Knowledge of the chemical form and bioavailability of selenium in mushrooms and other plant foods is necessary in order to assess the value of the foods as nutritional sources of selenium.

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