The Effect of Thermal and Non Thermal Food Processes and Cooking Method in some Essential Mineral Contents in Mushroom (*Agaricus bisporus*) in Iran

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**ABSTRACT:** This research builds upon: Determination, evaluation and comparing major essential minerals and trace elements contents (Iron, copper, zinc, manganese, Calcium, Magnesium and selenium) as nutritional value in mostly consumed brands of packaged mushrooms (*Agaricus bisporus*) samples purchased from Tehran markets in different states (sliced and conventional) and investigation the Effect of different cooking method (raw, cooked, fried, microwaved), in these minerals. 540 samples from the most famous packaged brands of *Agaricus bisporus* (white) were purchased in Tehran, Iran in 2013 in 2 consecutive months from January till August. Results indicated that the mean content of Zn, Cu and Fe in all samples has been increased by fried method while decreased significantly by microwaved method (p < 0.01). Magnesium and Calcium contents in raw state are the highest amount while heating by microwaved reduced these essential elements (p < 0.03). Selenium contents in cooked and fried method respectively show the highest and microwaved the lowest point. This investigation revealed that Thermal and Non Thermal Food Processes in *Agaricus bisporus* affected meaningfully the essential and trace mineral contents (Zn, Fe, Cu, Mg, Mn, Ca and Se). Despite of other studied elements zinc content in mushroom samples in raw state was less than microwaved condition.

**Keywords:** Mushroom, Essential Mineral Contents, Cooking Method, *Agaricus bisporus*.

**INTRODUCTION**

Cultivated mushrooms have higher protein contents and minerals, low in fat and rich in B vitamins, vitamin D, vitamin K and sometimes vitamins A and C (Saiqa et al., 2008; Sanmee et al., 2003; Alector 1995; Manzi et al., 2001; Mattila et al., 2001; Yildiz et al., 1998). In Asian countries, the fresh substrates normally containing straw, poultry litter, urea, and limestone are piled up outside for 15-25 days (Kim et al., 2011). The methods used for mixing wheat straw, animal litter, and limestone to make mushroom substrates are common among Asian countries (Kim et al., 2011; Bakshi, and Langar. 1991; Riahi et al., 1991). Mushrooms are one of the most valuable nutritional sources, low in calories but high in minerals, vitamins and vegetable proteins. Since ancient times mushrooms have been consumed by humans not only as a part of the normal diet but also as a delicacy because they have highly desirable taste and aroma (Kurbanoglu and Algur 2002). Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids, and minerals (Kalac 1991). They contain an especially high amount of B vitamins and potassium and other mineral elements. Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (Breene 1990; Kavyani et al., 2012). Mushrooms and its different derivatives contain a variety of active substances like ergothionine (Dubost and Beelman 2007; Kasuga et al., 1995), phenolic antioxidants, variegatic acid and dibiviquinone (Manzi et al., 1999). Fruit bodies of mushrooms are appreciated, not
only for texture and flavor but also for their chemical and nutritional characteristics (Sanmee et al., 2003; Nilanjana 2005). Fruiting body of mushrooms consists of cap (pileus) with a spore-forming part (sporohore) and stipe (stem, stalk). Mushroom uptake heavy metals from a substrate via spacious mycelium. The proportion contents originating from the atmospheric depositions seems to be less importance due to the short lifetime of a fruiting body, which is usually 10-14 days (Masamba and Kazombo-Mwale, 2010). Consumption of food with high contents of heavy metals can cause acute or chronic poisoning. A long-term exposure to heavy metals may result in cancer. As the consumption and cultivation of mushrooms has been increased remarkably in recent years in Iran due to the high price of red meat, fish and other proteins, this research builds upon:

- Determination and comparing essential mineral contents as an nutritional value in mostly consumed brands of packaged mushrooms (Agaricus bisporus) samples purchased from Tehran markets in different states (sliced and conventional).
- Investigation the Effect of cooking method (raw, cooked, fried, micro waved), in mineral contents in Mushroom samples.
- Determination the effect of seasons on the level of mineral contents in famous brands of packaged mushrooms sold in Tehran markets due to find probable reasons of variations.

MATERIALS AND METHODS

Sampling method

Mineral contents in 540 samples from the 6 most famous brands of Agaricus bisporus (white) were purchased in different states: sliced packaged (sliced fresh mushroom packaged) and conventional packaged (whole fresh mushroom packaged), in all different weight package available in market from creditable market in Tehran, Iran in 2013 in 2 consecutive seasons of winter and spring. Conventional & sliced mushroom samples purchased at the same day. Sampling was replicated twice within each month at intervals of two weeks. Due to this Descriptive Study the effect of cooking method, samples were studied in 4 different conditions: raw, cooked, fried, micro waved. Samples were randomly purchased for analysis and analyzed according to standardized international protocols by wet digestion method (AOAC 1989). All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines.

Preparing method:

The purchased fresh packaged samples were freed from foreign materials. Approximately 500 g of each brand of mushroom was washed firstly with tap water in order to remove sand and dirt and each mushroom sample rinsed with 300-350 ml deionized water and was divided into 4 portions and then followed by the procedure. One was retained fresh (raw), while the second portion of 100 gram was cooked by boiling deionized water. The boiling process was done according to the each kind of sample, which was approximately about 5 minutes for conventional samples and 3 minutes for sliced ones. The third portion of 100 gram was put about 3 minutes in olive oil preheated to 180 C\(^0\) till both sides of mushroom blushed. For preparing micro waved samples 100 gram of mushroom was cooked on high for 2 minutes for sliced samples and 3 minutes for whole mushrooms.

Zinc, Manganese, Copper Determination

For Zinc, Manganese, Copper and Selenium concentration 50 gram of each prepared mushroom sample was weighed and oven-dried at 50 C\(^0\) to a constant weight. Each oven-dried sample was ground in a mortar until it could pass through a 60 mesh sieve. The samples were stored in clean, dry, high density polyethylene bottles of 100 ml capacity with screw caps. Finally 5 gram of dried sample was weighed precisely on electronic balance (Shimadzu LIBROR AEX 200G). The samples were put in a 100 ml digestion flask and 20 ml of digestion mixture comprising of concentrated HNO3(65%) Merck and hydrochloric acid (70 %) Merck in the ratio of 3:1 was added to it and heated on a hot plate in the fuming chamber. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). This method has been followed in 4 stages for raw, cooked, fried, micro waved samples.

Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents (AOAC 1998). The flasks were firstly heated slowly and then vigorously till a white residue is obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO3 in a volumetric flask. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame, using at least five standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis (ORA LABORATORY MANUAL, 2013).
Iron Determination
The aliquot was passed through the atomic absorption spectrophotometer to read the iron concentration. Standards were prepared with a standard stock of 10 mg/L using ferrous ammonium sulphate where 3 - 60 ml of iron standard solution (10 Mg/L) were placed in stepwise volumes in 100 ml volumetric flasks. 2 ml of hydrochloric acid were added and then brought to the volume with distilled water. The concentration of iron in the aliquot was measured using the atomic absorption spectrophotometer in mg/L. The whole procedure was replicated three times.

Calcium and Magnesium Determination
5 ml of the aliquot were placed in a titration flask using a pipette and diluted to 100 ml with distilled water and subsequently 15 ml of buffer solution, ten drops of Eriochrome black T indicator and 2 ml of triethanolamine were added. The mixture was titrated with Ethylene-Diamine-Tetra-Acetate (EDTA) solution from red to clear blue (Masamba and Kazombo-Mwale, 2010).

Selenium Determination
Stock standard solutions for selenium were 1000 μg/mL solution. All reagents and standards were of analytical grade (Merck, Germany). The palladium matrix modifier solution was prepared by the dilution (10 g/L) Pd(NO₃)₂ and iridium AA standard solution, 1000 g/mL in 20% HCl, 0.1% V/V nitric acid prepared by dilution trace pure 65% nitric acid and 0.1% Triton X-100 were used. Doubly distilled water was used in all operations. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan). The analyze performed according by Analytical Method ATSRD (ATSDR, 2013).

Statistical Method
Seasonal differences on the basis of the type of mushroom and cooking method were determined by student t-test. Seasonal changes were calculated by one way Anova and for analysis of the role of multiple factors univariate analysis was used by SPSS 17. Probability values of <0.05 were considered significant.

RESULTS AND DISCUSSION

The results were determined as mean ± SD of three replicates in each test. All amounts reported as dried weight. Results revealed that even in the same month & same time of studying mushrooms there was a wide range of element contents in different brands of conventional and sliced samples. In figure 1 the wide range of selenium contents in the 6 studied brands is demonstrated.

Se content in food and beverages varies in different parts of the world from country to country, because its level in soil changes with native substrate, climatic conditions and vegetation cover. The Se content of animal products reflects the Se levels in their dietary intake (Barclay, MacPherson et al., 1995; Sirichakwal, Puwastien et al. 2005; Navarro-Alarcon and Cabrera-Vique, 2008).

Most plants do not have the ability to accumulate large amounts of Se (concentrations rarely exceed 100 μg/g, dry weight.)

![Figure 1. The mean level of Selenium contents (mg/kg DW) in 6 famous brands studied in 2 seasons of winter and spring 2013](image-url)
The results of Copper, Zinc, and Iron mean contents in 540 samples of raw, cooked, fried and micro waved mushroom samples in conventional and sliced forms from 6 creditable and best sellers of packaged mushroom (Agaricus bisporus) in Tehran, Iran market in two seasons of winter and spring are shown in Figure 2. Results indicated that the mean content of Zn, Cu and Fe in all samples has been increased by fried method while decreased significantly by microwaved method (p< 0.01). In these elements contents statistically meaningful differences were found in different brands (p< 0.03). Copper is involved in the activity of many enzymes and metabolic functions. It is necessary for the growth and maintenance of bones and is involved in the production of red blood cells, connective tissue and in metabolism of fats (Guide to Naturopathy edition by Geddes & Grosset 1999). Iron is an essential element for human beings and animals and is an essential reported by component of haemoglobin. It facilitates carbohydrates, protein and fat to control body weight, which is very important factor in diabetes (Ullah et al., 2012). Iron is necessary for the formation of haemoglobin and also plays an important role in oxygen transfer in human body and Low iron content causes gastrointestinal infection, nose bleeding myocardial infection (Ullah et al., 2012; Moses et al., 2012). Zinc is an essential trace element and foetus. plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of sensitivity and it cause loss of sense of touch and smell (Jabeen et al., 2010; ziarati, 2012; Moses et al., 2012). Zinc deficiency is common in people suffering from Chrohn’s disease, hypothyroidism and gum disease, and probably plays a part in susceptibility to viral infections and diabetes mellitus. It can be beneficial in the treatment of viral infections, including those of AIDS, prostate gland enlargement, rheumatoid arthritis, healing of wounds, acne, eczema and stress(Guide to Naturopathy edition by Geddes & Grosset, 1999).

The study indicated that size of samples has an effect on its essential and trace elements content as mostly in large mushrooms the Zn, Fe and Se content was less. Large size of conventional and sliced had significantly (p<0.01, p<0.05 respectively ) lower . A highly significant, although low, positive correlation ( r= 0.53, p=0.01, n=300) was found between Iron and Manganese contents of the conventional mushroom samples, compared to a non-significant and much lower correlation between the two variable in the sliced samples.

Figure 2. Comparing Zn, Fe and Cu contents (mg/kg DW) due to the state of cooking methods in Consumed Packaged Mushroom (Agaricus bisporus)

Figure 3. Comparing Mg and Ca contents (mg/kg DW) due to the state of cooking methods in Consumed Packaged Mushroom (Agaricus bisporus)
The mean level of Magnesium and Calcium and effects of cooking method has been presented in figure 3. There were significant differences in Magnesium and Calcium content in various methods of cooking (p<0.03). Magnesium and Calcium contents in raw state are the highest amount while heating by microwaved reduced these essential elements (p<0.03).

Selenium contents in cooked and fried method respectively show the highest and microwaved the lowest point. Microwaving method reduced the Selenium and Magnesium levels significantly in comparison by other thermal methods (p<0.03). The mean level of Se and Mn and effects of cooking method has been presented in figure 4. Results shows no significant differences of the level of mineral contents in famous brands of packaged mushrooms sold in Tehran markets by different seasons and months of studding.

![The Mean Contents of Mineral Contents (mg/kg DW) in Mushrooms in different states of cooking methods](image)

Figure 4. Comparing Se and Mn contents (mg/kg DW) due to the state of cooking methods in Consumed Packaged Mushroom (Agaricus bisporus)

**Results of the effect of cooking method, thermal and non thermal Process**

The accumulation of trace and essential mineral elements is strongly affected by the chemical composition of the substrate of compost that mushrooms get their nutrient. Geographical Condition of Cultivation may cause high content of lead. The results of this research revealed that particularly, the ranges of Cu, Fe, Mn and Zn levels tended to be higher in fried state while Mg and Ca contents are higher in raw state. Due to the effect of thermal and non thermal food processes and cooking methods, the levels of the mineral elements have been measured as follows:

**Se content**: Cooked > Fried > Raw > Micro

**Cu and Mn contents**: Fried > Cooked > Raw > Micro

**Fe content**: Fried > Raw > Cooked > Micro

**Mg and Ca contents**: Raw > Fried > Cooked > Micro

**Zn content**: Fried > Cooked > Micro > Raw

This research indicates that termal processes increase the level of Cu, Mn, Se, Fe and Zn while reduct Mg and Ca contents. However, the results obtained are comparable with some of the published articles and studies for other fruits and vegetables. Meta-analysis and more studies are suggested for the investegation on effects of different food processing techniques on mineral essential and trace elements levels on mushrooms, vegetables and fruits.

**REFERENCES**


