The Effect of cooking method in Potassium, lead and Cadmium Contents in Commonly Consumed packaged mushroom (Agaricus bisporus) in Iran

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ABSTRACT: 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 heavy metal contents (Lead and Cadmium) and potassium as an nutritional value in mostly consumed brands of packaged mushrooms (Agaricus bisporus) samples purchased from Tehran markets in different states (sliced and conventional) and comparing them by European Standard: EC No.466/2001 and investigation the Effect different seasons and also cooking method (raw, cooked, fried, micro waved), in Potassium, lead and Cadmium Contents in Mushroom samples. 540 samples from the most famous packaged brands of Agaricus bisporus (white) were purchased in Tehran, Iran in 2013 in 2 consecutive seasons of winter and spring. Results show that the content of Cadmium and Lead in 17.77% and 46.66% samples were over respectively. The mean content of potassium in all samples has been increased according to the cooking methods respectively: Micro waved <Raw< Sliced< Fried< Cooked, but for the mean level of heavy metals (lead and cadmium) content ranking from low to high concentration: Raw< Cooked< Fried< Micro < Sliced. The highest concentration of Lead was detected in sliced mushroom samples in winter season.

Keywords: Mushrooms, Lead, contamination, Agaricus bisporus, cooking method

INTRODUCTION

The mushroom is not a member of the plant kingdom as it evolved at a different time to plants and does not belong in either the plant or animal kingdoms. Being in a different biological kingdom helps explain why mushrooms have a different nutrition profile to fruits and vegetables, being particularly high in B vitamins and some minerals (Mushrooms and Health, 2010).

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). Most mushrooms have high protein content, usually around 20-30% by dry weight. This can be useful for vegetarians or anyone looking to increase the protein content in their(www.mushroom-appreciation.com). They
contain an especially high amount of B vitamins and potassium. Potassium’s role as an essential mineral is well established. Potassium helps maintain normal heart rhythm, fluid balance, muscle, and nerve function. The Dietary Approaches to Stop Hypertension (DASH) Trial demonstrated that an eating pattern which was rich in fruits and vegetables, and low fat dairy products, provided benefits for blood pressure (Obarzanek et al., 2001)) and blood cholesterol levels (Harsha et al., 2004). 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 ergothioneine (Dubost et al., 2007; Kavyani et al., 2012 ), phenolic antioxidants, variegatic acid and dibiviquinone (Kasuga et al., 1995). Effective compounds, present in the mushrooms possess antioxidant, antibacterial, immuneenhancing, and stress reduction activities (Dalloul and Lilhehoj, 2006; Dalloul et al., 2006). Recently, it has been reported that the combined use of Chinese herbal and mushroom extracts can operate as alternatives to antibiotic growth promoters in broiler chicken (Guo et al., 2004a,b; kavyani et al., 2012). (Also and Giannenas et al., 2010) observed the beneficial influence of Mushroom (Agaricus bisporus) on broilers performance and tissue antioxidant-protective activity.

Fruit bodies of mushrooms are appreciated, not only for texture and flavor but also for their chemical and nutritional characteristics (Maniz et al., 1999; Sanmee et al., 2003). Fruiting body of mushrooms consists of cap (pileus) with a spore-forming part (sporophore) 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 (Nilanjana, 2005).

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. Heavy metals such as cadmium and lead are important environmental pollutants, particularly in few studies have been reported that the contents of these toxic elements in food stuffs were higher than permissible levels (Anbari et al., 2011; ziarati et al., 2013 a; ziarati et al., 2013b).

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 heavy metal contents (Lead and Cadmium) and potassium as an nutritional value (Mushrooms contain more potassium than most other fruit and vegetables) in mostly consumed brands of packaged mushrooms (Agaricus bisporus) samples purchased from Tehran markets in different states (sliced and conventional) and comparing them by European Standard: EC No.466/2001.
- Investigation the Effect of cooking method (raw, cooked, fried, micro waved), in Potassium, lead and Cadmium Contents in Mushroom samples.
- Determination the effect of seasons on the level of heavy metals in famous brands of packaged mushrooms sold in Tehran markets due to find probable reasons of contamination.
- Assess the associated health risk posed to the Tehran population through exposure to lead and cadmium in mushrooms.

**MATERIALS AND METHODS**

**Sampling method**
Potassium, Cadmium and Lead 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 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.

For heavy metal analyses 50 gram of each prepared mushroom sample was weighed and oven-dried at 50 °C 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 LIBOR 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 for heavy metals: Pb and Cd, using at least five standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis (ORA LABORATORY MANUAL, 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. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University. The results of Cadmium and Lead 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 bisporu) in Tehran, Iran market in two seasons of winter and spring are shown in Figure 1.

Results revealed that even in the same season & same time of studying mushrooms there was a wide range of heavy metal contents in different brands of conventional and sliced samples. In table 1, this wide range is represented. The mean level of Cadmium content was lower than acceptable level (EU 466/2001) (2.0 mg/kg dry weight) in all samples except brand 2. Results show that the content of Cadmium and Lead in 17.77% and 46.66% from 6 brand samples were over respectively.

**Table 1. The Mean contents of Lead and Cadmium (mg/Kg DW) in all Conventional mushrooms packaged studied from different brands purchased from Tehran Market, 2013.**

<table>
<thead>
<tr>
<th>Conventional Agaricus bisporus packaged studied Brands in Tehran,Iran</th>
<th>Mean level of Lead content (mg/kg DW) ± SD</th>
<th>Mean level of Cadmium content (mg/kg DW) ± SD</th>
<th>Mean level of Potassium content (mg/kg DW) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.8102 ± 0.6002</td>
<td>0.4067 ± 0.1786</td>
<td>275.3338 ± 1.4890</td>
</tr>
<tr>
<td>2</td>
<td>8.8645 ± 1.8299</td>
<td>3.6368 ± 0.9655</td>
<td>269.1182 ± 1.4470</td>
</tr>
<tr>
<td>3</td>
<td>1.2112 ± 0.2315</td>
<td>0.3329 ± 0.0620</td>
<td>308.9549 ± 1.6592</td>
</tr>
<tr>
<td>4</td>
<td>2.7471 ± 0.8361</td>
<td>0.7812 ± 0.1934</td>
<td>280.1084 ± 1.4646</td>
</tr>
<tr>
<td>5</td>
<td>1.4235 ± 0.3416</td>
<td>0.4707 ± 0.1033</td>
<td>343.9974 ± 1.3066</td>
</tr>
<tr>
<td>6</td>
<td>4.0484 ± 0.3970</td>
<td>1.1521 ± 0.4832</td>
<td>283.0551 ± 1.4722</td>
</tr>
</tbody>
</table>

*SD= Standard Deviation
The majority of samples from the 2 most famous brands in Tehran market had detectable levels of lead much more above the permissible limit while the other ones especially less famous brands had lower than it. In the heavy metal and potassium contents, statistically meaningful differences were found in different brands. The study indicated that size of samples has an effect on its heavy metal content as mostly in large mushrooms the heavy metal content was less. Large size of conventional and sliced had significantly (p<0.01, p<0.05 respectively) lower lead and cadmium levels than small size in winter season. A highly significant, although low, positive correlation (r= 0.55, p=0.01, n=200) was found between lead and cadmium contents of the conventional mushroom samples, compared to a non-significant and much lower correlation between the two variables in the sliced samples. The mean level of potassium and effects of cooking method has been presented in figure 2. There were significant differences in potassium content in various methods of cooking (p<0.01).
**Results of the effect of cooking method**

The mean values of Lead and Potassium content between raw and cooked, raw and fried, raw and micro waved samples were analyzed by one – way Analysis of variance (ANOVA). The difference was considered statistically significant at the level of p<0.01. The response of mushroom samples to the experimental cooking method was different for the examined elements. Results revealed that mean content of potassium in all samples would be increased according to the cooking methods respectively: Micro waved <Raw<Sliced<Fried<Cooked, but for the mean level of heavy metals Lead and cadmium content ranking from low to high concentration: Raw< Cooked< Fried< Micro < Sliced.

The highest concentration of Lead was detected in mushroom samples in winter season and for Cadmium content although in all studied samples in 5 methods of cooking were less than permissible level set by EC466/2001 and no significant differences have been observed, but in sliced form the maximum contamination has been observed.

**Risk Assessment**

The daily intake of heavy metals through the consumption of the mushroom tested should be calculated according to the given equation:

\[
\text{Daily intake of heavy metals (µg/day)} = [\text{Daily vegetable consumption} \times \text{mushroom heavy metal concentration}]
\]

Due to have limited data deal with the daily intake in Iranian diet nowadays especially due to inflation and rising meat, fish and poultry prices and no data of chemical forms of bound metals and their bioavailability in man and the factors affecting the potential risk in human nutrition, assessing of the health risk from mushroom consumption has not been yet possible.

But this study proved that Consuming Agaricus bisporus more than 65 gram in a day even in the raw state that has the lowest content of heavy metals is risky and can be lead to health hazards while for intake calculations, usually 300 g of fresh mushrooms per meal is assumed (Kalac et al., 2004). Some people are more at risk, including pregnant women, the elderly, children and vegetarians. Food poisoning by Lead can also rise to other long-term illnesses and symptoms.

**CONCLUSION**

The accumulation of heavy metals and potassium 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. Chemical contaminations may occur in mushroom from various sources. As the use of pesticides, such as insecticides, fungicides or herbicides, has become an integral part of modern agriculture to increase crop yields and quality by controlling various pests, diseases and weeds, screening their residues should be done. The results revealed that particularly, the ranges of lead levels tended to be higher in sliced samples especially in the winter. Therefore the source of lead is probably related to the seasons, air pollution and the time of exposure of mushrooms to the environment due to environmental pollution. There is significant difference in the levels of lead in the quality of samples group analyzed. In our study, the major samples purchased in winter from the most famous brand in Tehran were found to have dangerous high lead contents especially in sliced form which likely had lower quality. They reasonably pose a health concern, resulting in strict monitoring and screening of their levels by national and international Alimentations Commission. Hence, analysis of relevant chemical contaminants is an essential part of food safety to ensure consumer safety. We suggest bioremediation or other green technologies such as phytoremediation for remediating and detoxifying contaminated soil and compost by heavy metals and other inorganic compounds considered detrimental to environmental health.
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REFERENCES


