Safety and Nutritional Comparison of Fresh, Cooked and Frozen Mushroom (Agaricus bisporus)

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ABSTRACT: As the consumption of different forms 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: Investigation the Effect of freezing process on the cooking method (raw, cooked, fried, micro waved), safety and nutritional value and the mean level of essential mineral contents in Mushroom samples. Mineral contents of 580 purchased samples from the 6 most famous packaged brands of *Agaricus bisporus* (white) were studied in 10 different states of thermal and non-thermal processing of conventional and sliced forms in all different weight package available in market from creditable market in Tehran, Iran in 2013 in 3 consecutive seasons of winter, spring and summer. Results revealed that although the mean contents of Zn, Cu and Fe in all samples has been increased by fried method but these contents in all samples decreased significantly by freezing method after being fried (p<0.01). In accordance of the result of mineral contents after freezing 4640 samples of raw-frozen, cooked-frozen, fried frozen and microwaved frozen finding that the mean level of mineral contents were reduced with freezing by approximately: 23.14% in raw-frozen, 19.55% in fried-frozen, 28.66% in cooked-frozen and 34.37% in microwaved-frozen samples.

Keywords: Mineral Contents, Frozen Mushroom, *Agaricus bisporus*

INTRODUCTION

A food can be regarded as functional if it has been scientifically proven to have special health benefits in addition to providing basic nutrients and nutritional benefits (Guizani and Sablani, 2007). Mushrooms are one of the most valuable nutritional sources, low in calories but high in minerals, vitamins and vegetable proteins. Mushrooms and its different derivatives contain a variety of active substances like ergothioneine (Dubost and Beelman 2007; Kasuga et al., 1995), phenolic antioxidants, variegatic acid and dibivquinone (Manzi et al., 1999). Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids, and minerals (ziarati et al., 2013c). Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (Breeene 1990; Kavyani et al., 2012). Fruiting bodies of mushrooms are appreciated, not only for texture and flavor (Ouzouni et al., 2009; Bano et al., 1992; Bano et al., 1993; Rajarathnam and Bano 1990; Lee et al., 2009; Sanmee et al., 2003; Nilanjana 2005) but also for agricultural production (Bano and Rajarathnam 1986; Bano and Rajarathnam 1988; Mallikarjuna et al., 2013). According to statistics, *A. bisporus* is the most widely cultivated edible mushroom (Chang and Miles 2004). *A. bisporus* is an excellent source of several essential amino acids, vitamins (B2, niacin and folate) and minerals (potassium, phosphorus, zinc and copper) (Manzi et al., 2001; Guizani et al., 2013). Fresh mushrooms have high water content, around 90%. The ash content of edible mushrooms ranges from 6% to 11% DW and contains a wide variety of minerals. They are also a good source of...
Minerals. The major mineral constituents are potassium (K), phosphorus (P), sodium (Na), calcium (Ca), magnesium (Mg) and selenium (Se). Copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo) and cadmium (Cd) make up the minor mineral constituents (Chang and Miles 2004; Cheung 2010). However, the commercial value of A. bisporus can be decreased or fully lost within few days if it is stored at ambient temperature. Along with its consumption as fresh, A. bisporus can be preserved in the dried, frozen and freeze-dried forms (Guizani et al., 2013). 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).

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., 2013a; ziarati et al., 2013b, ziarati et al., 2013c). 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 of different forms of mushrooms has been increased remarkably in recent years in Iran due to the high price of red meat, fish and other proteins (ziarati et al., 2013c), this research builds upon: Investigation the Effect of freezing process on the cooking method (raw, cooked, fried, micro waved), safety and nutritional value and the mean level of essential mineral contents in Mushroom samples. While the majority of samples from the 2 most famous brands in 2012 and 2013 in Tehran market had detectable levels of lead much more above the permissible limit (ziarati et al., 2013c), in this study the effect of freezing method on the level of Lead and Cadmium as well as other elements have been determined.

**MATERIALS AND METHODS**

**Sampling method**

Mineral contents of 580 purchased samples from the 6 most famous packaged brands of Agaricus bisporus (white) were studied in different states: raw, raw-frozen, fried, fried-frozen, cooked, cooked-frozen, microwaved, microwaved frozen of conventional and sliced forms in all different weight package available in market from creditable market in Tehran, Iran in 2013 in 3 consecutive seasons of winter, spring and summer. 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 freezing on cooking method, 4640 samples were studied in 10 different conditions: raw /raw-frozen, cooked/cooked-frozen, fried/fried-frozen, microwaved/microwaved-frozen, sliced/sliced-frozen which means all samples were studied according to the thermal processing and then all of them were frozen in freezer for 3 days at -20°C. 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. After studied on the effect of thermal processing they all were frozen in -20°C in freezer for 3 days.

**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 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.
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).

Lead and Cadmium Determination

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 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 10 stages for raw/ raw-frozen, cooked/ cooked-frozen, fried/ fried-frozen, microwaved / microwaved and sliced/sliced-frozen 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.
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

Results

All samples concentrations were reported as mg/kg dry weight of materials. The results were determined as mean ± SD of three replicates in each test. The elements Pb, Cd, Zn, Cu, Fe, Mn and Se were determined by FAA spectrometry. Fe and Zn were determined by both analytical methods. The results of Copper, Zinc, Manganese and Iron mean contents in the samples of 10 different states of thermal and non-thermal processing after being frozen for 3 days at -20°C from packaged mushroom (Agaricus bisporus) are shown in table 1.

Table 1. The mean contents of mineral elements of Fresh, Cooked and Frozen Mushroom (Agaricus bisporus) samples

<table>
<thead>
<tr>
<th>Thermal and non-thermal processing</th>
<th>Ca (mg/kg DW)</th>
<th>Mg (mg/kg DW)</th>
<th>Zn (mg/kg DW)</th>
<th>Fe (mg/kg DW)</th>
<th>Cu (mg/kg DW)</th>
<th>Mn (mg/KgDW)</th>
<th>Se (mg/Kg DW)</th>
</tr>
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<tbody>
<tr>
<td>Raw</td>
<td>879.11</td>
<td>1434.09</td>
<td>125.77</td>
<td>66.24</td>
<td>56.44</td>
<td>5.77</td>
<td>3.11</td>
</tr>
<tr>
<td>Raw-Frozen</td>
<td>802.12</td>
<td>1420.31</td>
<td>103.44</td>
<td>54.87</td>
<td>52.1</td>
<td>5.2</td>
<td>3</td>
</tr>
<tr>
<td>Cooked</td>
<td>805.16</td>
<td>1564.01</td>
<td>153.44</td>
<td>62.11</td>
<td>59.4</td>
<td>6.43</td>
<td>3.54</td>
</tr>
<tr>
<td>Cooked-Frozen</td>
<td>734.88</td>
<td>1511.91</td>
<td>121.89</td>
<td>53.39</td>
<td>55.33</td>
<td>6.04</td>
<td>3.22</td>
</tr>
<tr>
<td>Micro</td>
<td>764.32</td>
<td>1328.37</td>
<td>133.09</td>
<td>60.32</td>
<td>42.11</td>
<td>4.39</td>
<td>2.77</td>
</tr>
<tr>
<td>Micro-Frozen</td>
<td>705.12</td>
<td>1276.35</td>
<td>116.17</td>
<td>48.72</td>
<td>32.06</td>
<td>3.36</td>
<td>2.03</td>
</tr>
<tr>
<td>Fried</td>
<td>834.66</td>
<td>1544.32</td>
<td>156.44</td>
<td>69.45</td>
<td>46.98</td>
<td>7.33</td>
<td>3.41</td>
</tr>
<tr>
<td>Fried-Frozen</td>
<td>799.89</td>
<td>1466.79</td>
<td>145.21</td>
<td>54.3</td>
<td>40.61</td>
<td>6.24</td>
<td>3.19</td>
</tr>
<tr>
<td>Sliced</td>
<td>854.81</td>
<td>1420.95</td>
<td>149.09</td>
<td>65.44</td>
<td>51.13</td>
<td>5.7</td>
<td>2.99</td>
</tr>
<tr>
<td>Sliced-Frozen</td>
<td>743.51</td>
<td>1410.92</td>
<td>108.95</td>
<td>56.71</td>
<td>47.21</td>
<td>5.05</td>
<td>2.63</td>
</tr>
</tbody>
</table>

The results of Magnesium and Calcium mean contents in all samples of studied mushrooms are shown in figure 1. Results indicated that Mg and Ca mean contents in all samples have been decreased significantly (p<0.03) by freezing method, while the effect of freezing in decreasing the mean content of essential and nutritional Calcium element in sliced mushrooms was more (p<0.005). A highly significant, although low, positive correlation (r = 0.51, p= 0.005, n= 430) was found between Zinc and copper mean contents of the conventional raw mushroom samples (whole part of fresh mushroom), compared to a non-significant and much lower correlation between the two variable in the sliced samples. The mean level of Copper, Zinc and Iron contents in samples of raw, raw-frozen, sliced, sliced-frozen, fried, fried-frozen, cooked, cooked-frozen, microwaved and microwaved-frozen in packaged mushrooms are shown in figure 2. Results revealed that although the mean contents of Zn, Cu and Fe in all samples has been increased by fried method but these contents in all samples decreased significantly by freezing method after being fried (p<0.01).

![Figure1. Comparing Magnesium and Calcium contents (mg/kg DW) in 10 different state of thermal and non-thermal processing in Consumed Packaged Mushroom (Agaricus bisporus)](image-url)
Selenium contents in cooked and fried method respectively show the highest and microwaved the lowest point in cooking method and after freezing, the microwaved-frozen samples in comparison by other frozen forms had the lowest level. The mean level of Se and Mn and effects of thermal and non-thermal processing method has been presented in figure 3.

Safety Comparison of Lead and Cadmium Contents

The mineral composition reflects on the grow conditions of the mushrooms, therefore in this study as the mean lead content of raw state is high similar observations were made from the thermal processing. Lead contents are dealt with the kind of thermal processing (p<0.03). The highest level of heavy metal lead are shown in sliced-frozen samples. Concentration of lead and cadmium in the studied packaged mushroom samples were mostly higher than permissible level of EC 466 (Ec466/2001) (table 2).

Table 2. The mean contents of Lead and Cadmium in Fresh, Cooked and Frozen Mushroom (Agaricus bisporus) samples

<table>
<thead>
<tr>
<th>Thermal and non-thermal processing</th>
<th>Pb(mg/kgDW)</th>
<th>Cd(mg/kgDw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>2.9212</td>
<td>0.7638</td>
</tr>
<tr>
<td>Raw-Frozen</td>
<td>2.9122</td>
<td>0.7543</td>
</tr>
<tr>
<td>Cooked</td>
<td>3.4836</td>
<td>1.1235</td>
</tr>
<tr>
<td>Cooked-Frozen</td>
<td>3.0053</td>
<td>1.1209</td>
</tr>
<tr>
<td>Micro</td>
<td>3.7274</td>
<td>1.1834</td>
</tr>
<tr>
<td>Micro-Frozen</td>
<td>3.7765</td>
<td>1.1768</td>
</tr>
<tr>
<td>Fried</td>
<td>3.6046</td>
<td>1.884</td>
</tr>
<tr>
<td>Fried-Frozen</td>
<td>3.4530</td>
<td>1.7530</td>
</tr>
<tr>
<td>Sliced</td>
<td>3.8506</td>
<td>1.3913</td>
</tr>
<tr>
<td>Sliced-Frozen</td>
<td>3.7867</td>
<td>1.3312</td>
</tr>
<tr>
<td>EC446</td>
<td>3</td>
<td>2</td>
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</table>
Discussion

This research indicates that heating process increase the level of Cu, Mn, Se, Fe, Zn and Cd and pb while freezing after cooking in different methods decrease the mean level of all studied elements.

In accordance of the result of mineral contents after freezing 4640 samples of raw-frozen, cooked-frozen, fried frozen and microwaved frozen finding that the mean level of mineral contents were reduced with freezing by approximately: 23.14% in raw-frozen, 19.55% in fried-frozen, 28.66% in cooked-frozen and 34.37% in microwaved-frozen samples. The accumulation of trace and essential mineral elements is strongly affected by thermal and heating processing. 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 investigation on the effects of different food processing techniques on the other minerals and trace elements levels on the other kind of edible mushrooms and vegetables and fruits.

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REFERENCES


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