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EFFECT OF THE ADDITION OF DRIED SHIITAKE (*LENTINULA EDODES*) TO CORN CRACKERS ON THEIR CHEMICAL COMPOSITION AND ABILITY TO BIND Fe(III) AND Zn(II) – A SHORT REPORT

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The aim of this study was to assess the chemical composition of corn crackers with a 10% and 20% addition of dried shiitake and to estimate the sorption of Fe and Zn by these products under conditions of pH values similar to those found in the human alimentary tract. Contents of protein, fat and ash were determined using standard analytical methods. Soluble and insoluble fractions of dietary fiber were assayed using the Asp's enzymatic method. Contents of minerals were analysed by atomic absorption spectrophotometry. Sorption of Fe and Zn was estimated under *in vitro* conditions (simulated to imitate those occurring in the alimentary tract), using buffer solutions with pH=1.8, pH=6.6, and pH=8.7 as dispersion media. The products were found to be characterised by high contents of fiber, Fe, Cu, Mg and K, and low contents of Na and Ca. A low ability to bind Fe and Zn of not more than 22% was observed in the analysed material. Experimental results obtained indicate that the products with the addition of dried shiitake could be used as a food additive; however, in order to promote them as a good source of microelements it is necessary to determine the sorption capacity of dried mushrooms in *in vivo* tests.

INTRODUCTION

Mushrooms have traditionally been used in the human diets, yet in the last years their cultivation has been increased. From the nutritional point of view, mushrooms are low in calories and fat, have a higher protein content than most vegetables, and are rich in vitamins (B, D) and minerals (K, P, Mg, Ca, Zn, Cu, Mn, Se) [Jansson & Kutti, 2004; Isiloglu *et al.,* 2001]. Cultivated mushrooms, especially shiitake (*Lentinulla edodes)*, due to their documented prebiotic properties and a relatively high nutritive value and contents of minerals are recommended in many countries as an addition to an everyday diet. However, the recommended introduction to diet of products with the addition of dried shiitake, particularly as a source of Fe and Zn requires a precise determination of contents of these elements and on the other hand – the sorption of microelements by these products. Many experiments indicate the possibility of lowering the level of mineral substances resulting from the intake of a diet rich in dietary fiber. On the basis of literature data, it is possible to put forward a hypothesis that metal binding capacity on fiber preparations depends on the source of dietary fiber, adsorbent type, processing method, pH and temperature of the medium, as well as on the metal whose sorption was investigated [Thompson & Weber, 1979; Stachowiak & Gawęcki, 1989; Stachowiak & Kubiak, 1990; Korczak *et al.*, 1995; Górecka & Stachowiak, 2002]. Thus, the aim of this study was to assess the chemical composition of the obtained products with added dried shiitake and to estimate the sorption of Fe and Zn by these products under pH conditions similar to those found in the human alimentary tract.

MATERIALS AND METHODS

Material

The material consisted of corn crackers with the addition of dried shiitake. Crackers were produced from maize flour (10% and 20% maize were substituted with dried mushrooms), margarine (16.6 g/100 g), milk (12 g/100 g) and water (21 g/100 g). Dried shiitake was produced from fruiting bodies grown on the beech sawdust substrate enriched with 20% ground wheat grain, inoculated with granular mycelium of shiitake cv. 'Sylvan 4080'. Incubation was carried out at the temperature of 25°C and relative humidity of 80-85% on average for 80 days. After the incubation the mushrooms were grown at 17-18°C and relative humidity of 85-90% on average for 12 days.

Chemical analysis

Contents of protein, fat, ash, carbohydrates, and energy value were determined using standard analytical methods [ICC – Standards, 1982; AOAC, 1995]. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined enzymatically according to Asp [1983]. Measurements of Fe, Zn, Cd and Pb were carried out according to the Polish Standard [PN-EN 14082, 2004]. The samples were dry-mineralized

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and determined using atomic absorption spectrophotometry in a SOLAR 939 apparatus by UNICAM LIMITED CAMBRIDGE. The content of Hg was analysed using methods described by Ludwicki [1990]. Analytical assays were performed in three simultaneous replications and the results were presented in grams per 100 g solids.

To determine the capacity of crackers to bind minerals Zn and Fe, use was made of the methodology based on a study by Stachowiak & Kubiak [1990], consisting in shaking product samples at the temperature of 37°C. The adsorbates used were solutions containing $ZnCl_2$ and $FeCl_3$ with the following concentrations of individual elements: Zn 25 μg/mL and Fe 30 μg/mL. The dispersing solutions were buffer solutions with pH=1.8, pH=6.6 and pH=8.7. Experimental conditions were selected to imitate those found in the human alimentary tract: the oral cavity (pH=6.6, shaking time of 7 min), the stomach (pH=1.8, shaking time of 135 min) and the duodenum (pH=8.7, shaking time of 60 min). Then, the sample was centrifuged at 5000 rpm for 10 min in order to separate supernatant. The sorbent was washed with buffer (respectively pH=1.8, pH=6.6 and pH=8.7). The shaking and centrifuging procedure was repeated 3 times, after which the sample was dried at 60°C until a constant weight has been obtained. The volume of sorption was defined as the percentage ratio of the content of the element bound by the product to the total amount of this element introduced to the system.

Statistical analysis

Statistical analysis was performed using ANOVA for factorial designs and differences between individual groups were analysed using the Tukey's HSD test with the application of a statistical software package Statistica 7.0 by StatSoft.

TABLE 1. Chemical composition of crackers with the addition of dried shiitake (*Lentinula edodes*).

The energy value (kcal) and contents of macrocomponents (g/100 g dry matter)		
	10% of dried shiitake	20% of dried shiitake
Energy value	543±1.47	546±4.30
Water	4.43±0.05[a]	6.41±0.12[b]
Protein	9.33±0.13	10.3±0.41
Fat	27.7±0.35	28.4±0.95
Carbohydrates	64.1±0.29	62.5±0.66
Ash	1.66±0.03	1.84±0.01
Soluble dietary fiber	5.36±0.54	4.89±0.15
Insoluble dietary fiber	11.6±0.13[a]	16.7±0.28[b]
Contents of minerals (mg/100 g dry matter)		
Fe	16.2±0.39	17.3±0.72
Cu	1.83±0.025[a]	2.72±0.04[b]
Zn	14.8±0.05[a]	19.7±0.38[b]
Mg	464±12.7	485±4.59
Ca	286±0.63[a]	304±12.4[b]
K	3455±114[a]	5657±91.8[b]
Na	787±26.2[a]	916±133[b]
Contents of toxic metals (mg/kg dry matter)		
Pb	0.000±0.000	0.000±0.000
Cd	0.166±0.0064[a]	0.326±0.0085[b]
Hg	0.0030±0.001	0.0020±0.0006

Significant difference between row means of each group is indicated by different superscript letters (Tukey's HSD test, $p<0.001$)

RESULTS

Table 1 presents energy value, contents of macrocomponents, fiber, minerals and toxic metals in products with a 10 and 20% addition of dried shiitake. No significant effect of dried mushrooms addition was found on changes in macrocomponent contents in the crackers. Both products tested were characterised by a high, although varying content of total dietary fiber (TDF). Its mean level for crackers with a 10% addition of dried shiitake was 16.7 g/100 g solids, while for crackers with a 20% addition of dried shiitake it was 21.6 g/100 g solids, at a lower content of soluble fraction in comparison to the insoluble fraction. The content of SDF ranged from 4.89 g/100 g dry matter (crackers with a 20% addition of dried mushrooms) to 5.36 g/100 g dry matter (crackers with a 10% addition of dried shiitake). The percentage portion of dried mushrooms added to products had a significant ($p<0.001$) effect on contents of insoluble fiber, the content of which in both crackers increased by approx. 40%. A significant ($p<0.001$) increase was also observed in the contents of Fe, Cu, Zn, K and Na along with a change in the portion of dried shiitake added to products. The products were characterised by high contents of Mg and K as well as by an advantageous ratio of sodium to potassium. No exceeded admissible levels of toxic metal contents (in dry mushroom: Pb – 2.00 mg/kg, Cd – 1.00 mg/kg, Hg – 0.05 mg/kg, in cereal products: Pb – 0.30 mg/kg, Cd – 0.05 mg/kg, Hg – 0.01 mg/kg) [Ordinance of the Ministry of Health, 2003] were recorded in the products, still Cd contents were relatively high.

The statistical analysis of results obtained for the ability to bind Fe(III) and Zn(II) by crackers with added dried shiitake showed that the level of binding of these metals was dependent ($p<0.001$) on the ambient pH level and percent addition of dried mushroom. The values of metal sorption were the lowest at pH=1.8, with binding of Fe and Zn by products at this pH not exceeding 3% (Figures 1 and 2). The sorption of Fe by crackers of not more than 22% were recorded at pH=6.6.

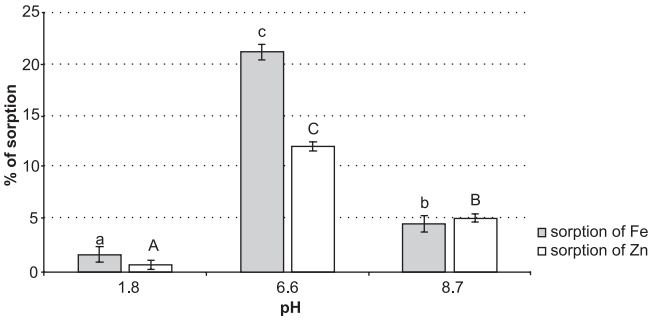

FIGURE 1. Sorption of iron and zinc by crackers with a 10% addition of dried mushroom (a, b, c – significant difference between sorption of Fe, $p<0.001$; A, B, C – significant difference between sorption of Zn, $p<0.001$).

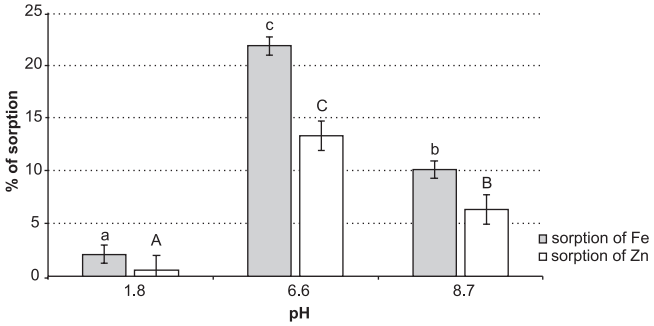

FIGURE 2. Sorption of iron and zinc by crackers with 20% addition of dried mushroom (a, b, c – significant difference between sorption of Fe, $p<0.001$; A, B, C – significant difference between sorption of Zn, $p<0.001$).

DISCUSSION

For several years, numerous studies have been presented in literature on nutrition that documented prebiotic properties of shiitake [Jansson & Kutti, 2004; Isiloglu *et al.*, 2001; Manzi & Pizzoferrato, 2000; Jeng-Leun *et al.,* 2002; Smith *et al.,* 2002; Reguła & Siwulski, 2007; Shimada *et al.,* 2002; Rajewska & Balasinska, 2004]. They have put special emphasis on high contents of biologically-active components exhibiting antitumour action, reducing LDL cholesterol level, lowering arterial blood pressure and influencing the immune system [Manzi & Pizzoferrato, 2000; Jeng-Leun *et al.,* 2002; Shimada *et al.,* 2002; Rajewska & Balasinska, 2004]. It is believed that these mushrooms may also be a good source of micro- and macroelements, such as K, Mg, P, Zn, Fe and Cu [Jansson & Kutti, 2004; Isiloglu *et al.,* 2001; Reguła & Siwulski, 2007; Breene, 1990; Kalac & Svoboda, 2000]. These mushrooms are easy to grow and cheap to produce. Although information on the nutritive value of shiitake mushrooms and their dried products is abundant in literature published worldwide, there are practically no comprehensive studies on foodstuffs prepared with these mushrooms, particularly information on the range of metal binding capacity of the analysed products, which may considerably reduce the availability of these microelements by the organism. This study is an attempt to elucidate these problems, on an example of crackers with a 10 and 20% addition of dried shiitake.

The products tested were characterised by a relatively high nutritive value. Their protein content was similar to the levels recorded in cereal products, while numerous authors suggest that shiitake protein is relatively readily available and that methionine is the amino acid limiting their nutritive value [Jansson & Kutti, 2004; Breene, 1990; Rajewska & Balasinska, 2004]. A high content of dietary fiber in the crackers, considerably exceeding values recorded in cereals, needs to be emphasized here. High contents of ash as well as Fe, K, Mg, P, Zn and Cu were recorded in the crackers, with the contents of these minerals significantly affected by the addition of dried shiitake to those products. Some authors suggested that food rich in dietary fiber may disturb mineral balance in the human organism through their adsorption [Korczak *et al.*, 1995; Stachowiak & Gawęcki, 1989; Górecka & Stachowiak, 2002; Harland, 1995]. Excessive adsorption of minerals is caused by the presence of phytates. To be good sources of minerals, products should not exhibit strong sorptive properties. In this study, the sorption of Zn and Fe was relatively high only at pH=6.6, which is consistent with observations made by other authors that most sorbents of different origin (dried apples; dried carrot; wheat, maize, soy and rice bran; oat glumes and cellulose) bind metals at pH=6.8, while along with increasing acidity of the solution a decrease in sorption is observed, and even at pH=0.65 the release of Cu, Zn and Fe is observed [Stachowiak & Kubiak, 1990; Korczak *et al.,* 1995; Thompson & Weber, 1979; Górecka & Stachowiak, 2002; Reguła & Siwulski, 2007; Stachowiak & Smigielska, 2004]. In the analysed crackers, the maximum sorption values were recorded at pH=6.6 for Fe, which is however a lower value in comparison to those reported by other authors [Stachowiak & Kubiak, 1990; Thompson & Weber, 1979; Stachowiak & Gawęcki, 1989]. Reguła & Siwulski [2007] conducted a study on dried shiitake mushroom and observed that values of metal sorption were the lowest at pH=1.8, whereas binding Zn and Fe by dried shiitake did not exceed 25%. Along with the increasing pH of the medium the metal binding capacity of dried mushroom increased. The strongest metal sorption capacity was found for dried shiitake in the medium with pH=8.7, with the highest value reported for Fe. Although dried shiitake has relatively strong abilities to bind metals, their addition to the product did not increase significantly the value of Fe and Zn sorption. A comprehensive elucidation of sorption capacity of these products might be obtained in *in vivo* studies.

CONCLUSIONS

Taking into consideration a relatively high nutritive value manifested in contents of protein, dietary fiber, Mg, K, Fe, Cu and Zn, as well as prebiotic properties suggested by other authors, the products obtained, *i.e.* corn crackers, may be recommended as a supplement to a traditional diet [Breene, 1990; Rajewska & Balasinska, 2004]. However on account of the possibility of accumulating heavy metals by mushrooms, the World Health Organization (WHO) [Hobbs, 2003] is advising to limit a weekly intake of fresh mushrooms to 250 g per person, which corresponds with the intake of 900 g of crackers with 10% addition of shiitake mushrooms per week. To promote the products with shiitake additives as a good source of microelements it would be necessary to determine their sorption capacity in *in vivo* studies.

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