

# Iron bioavailability from cereal products enriched with *Pleurotus ostreatus* mushrooms in rats with induced anaemia

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## Abstract

**Introduction and objective.** Oyster mushroom *Pleurotus ostreatus* is good source of iron. However, there is a limited data concerning bioavailability of iron from oyster mushroom and also cereal products containing this mushroom. The aim of this study was to assess bioavailability of iron from products with an addition of *Pleurotus ostreatus* in male rats with anaemia.

**Material and methods.** Investigations were conducted in two stages. In the first stage iron deficiency was developed in rats. For this purpose 6 weeks old 36 male Wistar rats were fed a AIN-93M diet deficient in iron and 6 males received a standard AIN-93M diet. In the second stage of the study the assessment of Fe bioavailability from cereal products enriched with dried *Pleurotus ostreatus*. After experiment the animals were killed and blood and heart, liver, spleen and kidneys were collected for biochemical tests.

**Results.** Feeding male Wistar rats supplemented with dried *Pleurotus ostreatus* mushrooms diets resulted in the restitution of the systemic Fe level, as manifested by an increase of the level comparable to the control group for: iron transferrin saturation rate, haemoglobin and mean corpuscular volume. Values of hematocrit, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in animals fed products supplemented with *Pleurotus ostreatus* were significantly higher compared to animals fed products with no Fe added. The highest MCV value was recorded when 20% of dried oyster mushrooms were added. Iron levels in the blood serum, the liver and kidneys in animals fed cereal products considerably exceeded values recorded at the beginning of the experiment and were similar to the control values.

**Conclusions.** Product may be a valuable source of iron in the nutrition of individuals with a deficiency of this element, first of all patients with absorption and metabolism disorders, but also may add variety to the traditional daily diet.

## Key words

minerals, blood, functional food, availability

## INTRODUCTION

Iron plays a significant role in the functioning of the organism in mammals because it participates in oxygen transport and storage (as a component of haemoglobin and myoglobin), electron transfer (in cytochromes), desaturation of fatty acids, tyrosine iodination (in thyroid peroxidase), prostaglandin synthesis, etc. This element also serves a primary role in energy metabolism, formation of erythrocytes, maintenance of heat balance, as well as humoral and cellular resistance [1]. Iron metabolism comprises several stages, i.e. absorption, transport and participation in metabolic processes and storage, mainly in hepatocytes and cells of the reticuloendothelial system [2, 3]. Inadequate iron supply with the diet leads first to a negative balance and depletion of reserves, followed by biochemical changes, reflecting the deficit of iron required for the production of haemoglobin, resulting in anaemia [4, 5, 6, 7].

The degree of iron absorption with food varies. Apart from the degree of organism saturation with this nutrient, an important role is played by the type of consumed product, the physical and chemical form of iron, and interactions between diet components. Factors enhancing bioavailability

and absorption of iron include low iron contents in the organism, the presence of meat in a meal, together with products with high contents of vitamin C, folic acid, amino acids (histidine, L-cysteine), copper, organic acids, including citric acid and lactic acid. In turn, factors reducing iron absorption are phytates, oxalates, tannins, polyphenols and phosphates, mainly in combination with calcium, as well as minerals such as manganese and zinc [7, 8, 9]. Some authors [7, 10] suggested that food rich in dietary fibre may disturb iron metabolism in the human organism by binding it. For products to be good sources of minerals they may not exhibit strong sorption properties [11].

There are two types of iron in food: haeme Fe(II), found in animal origin products, and non-haeme Fe(III), contained mainly in plant origin products (cereal products and in dry seeds of legumes and in certain leafy vegetables). Bioavailability of haeme iron may be as high as 30%, while of non-haeme iron it is around 1 – 8%. Thus, many studies are being conducted aimed at an improvement of non-haeme iron bioavailability thanks to the enrichment of food with components increasing its absorption.

Functional food enriched with iron is an essential element in the control of anaemia and offers good prospects for the future in developing countries, particularly in the poorest regions of the world. Such food may also be a valuable supplementation of daily diet in feeding different population groups, including vegetarians. An alternative for foodstuffs enriched with a synthetic form of iron may be provided by a

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product containing a natural material rich in this nutrient. The oyster mushroom *Pleurotus ostreatus* is a good source of iron, and is well-documented by many researchers [11, 12, 13, 14, 15, 16]. However, there is a limited data concerning the bioavailability of iron from oyster mushroom *Pleurotus ostreatus* and other products containing this mushroom.

## OBJECTIVE

The aim of this study was to assess the bioavailability of iron from products with an addition of *Pleurotus ostreatus* in male rats with anaemia. In order to recommend the cereal products enriched with dried oyster mushroom as a good source iron it is necessary to determine the bioavailability of iron from this product. A cereal product naturally enriched with iron of good bioavailability might be add to the functional products, particularly for vegetarians.

## MATERIAL AND METHODS

**Animals and diets.** Investigations were conducted in two stages. In the first stage iron deficiency was developed in rats bred. For this purpose 6 weeks old 36 male Wistar rats were fed for 28 days a AIN-93M diet deficient in iron (Tab. 1). At the same time, a control group of 6 males received a standard AIN-93M diet [17]. After 28 days, blood was collected from the tails of 12 animals (6 from each group), and Fe determined in haemoglobin, haematocrit and Fe concentration in blood serum. After the Fe deficiency was detected, the animals were killed and blood, heart, liver, spleen and kidneys were collected for analyses.

**Table 1.** Composition of experimental AIN-93M diets

Components	Diets	
	Fe deficient AIN-93M	Control AIN-93M
Casein (g·kg <sup>-1</sup> )	200	200
Soybean oil (g·kg <sup>-1</sup> )	70	70
Wheat starch (g·kg <sup>-1</sup> )	530	530
Sucrose (g·kg <sup>-1</sup> )	100	100
Potato starch (g·kg <sup>-1</sup> )	50	50
Vitamin premix (g·kg <sup>-1</sup> )	10	10
Mineral premix (g·kg <sup>-1</sup> )	40 <sup>a</sup>	40

<sup>a</sup> Mineral premix without Fe

In the second stage of the study, the Fe bioavailability from cereal products enriched with dried *Pleurotus ostreatus* was assessed. For this purpose, animals with Fe deficiency received test diets for 26 days: 12 rats (6 in each of the groups) were fed cookies containing 10% and 20% of dried oyster mushroom (Tab. 2), and 18 males (6 in each of the groups) were fed a control diet: cookies without dried mushrooms, Fe deficient AIN-93M diet and a complete semi-synthetic AIN-93M diet. Cereal cookies (CB0) were produced from corn flour, while for CB1 and CB2, 10% and 20% of the flour was replaced with ground dried *Pleurotus ostreatus* mushrooms. Products were baked at 180°C for 15 min. The composition and nutritive value of the cookies are given in Table 2. The composition and analytically assayed nutritive value of semi-synthetic diets and cookies are presented in Table 3.

**Table 2.** Composition of cookies

Components	Diets		
	CO	CB1	CB2
Corn flour (g·kg <sup>-1</sup> )	650	550	500
Oyster mushroom (g·kg <sup>-1</sup> )	0	60	100
Olive oil (g·kg <sup>-1</sup> )	90	90	90
Margarine (g·kg <sup>-1</sup> )	160	160	160
Milk (g·kg <sup>-1</sup> )	160	160	160

CB1, CB2 – Cereal products with the 10% and 20% *Pleurotus ostreatus*  
CO – Cereal products without the addition *Pleurotus ostreatus*

**Table 3.** Chemical composition of diets (mean ± SD)

	Fe deficient AIN-93M	Control AIN-93M	CB1	CB2	CO
Energy (MJ·kg <sup>-1</sup> )	18.3 ± 1.6 <sup>a</sup>	18.4 ± 2.0 <sup>a</sup>	20.1 ± 2.5 <sup>a</sup>	19.6 ± 1.8 <sup>a</sup>	20.7 ± 1.1 <sup>a</sup>
Dry matter (g·kg <sup>-1</sup> )	920.2 ± 2.5 <sup>a</sup>	930.0 ± 1.2 <sup>a</sup>	940.5 ± 1.5 <sup>a</sup>	950.0 ± 2.1 <sup>a</sup>	950.2 ± 1.2 <sup>a</sup>
Protein (g·kg <sup>-1</sup> )	100.0 ± 10.3 <sup>b</sup>	100.0 ± 4.2 <sup>b</sup>	50.00 ± 1.0 <sup>a</sup>	80.00 ± 1.3 <sup>a</sup>	60.00 ± 0.3 <sup>a</sup>
Fat (g·kg <sup>-1</sup> )	110.0 ± 1.3 <sup>a</sup>	120.0 ± 4.2 <sup>a</sup>	230.0 ± 2.3 <sup>b</sup>	200.0 ± 0.4 <sup>b</sup>	240.0 ± 1.3 <sup>b</sup>
Carbohydrates (g·kg <sup>-1</sup> )	740.5 ± 2.3 <sup>a</sup>	730.0 ± 3.2 <sup>a</sup>	630.7 ± 5.9 <sup>a</sup>	640.5 ± 8.6 <sup>a</sup>	630.8 ± 5.5 <sup>a</sup>
Crude ash (g·kg <sup>-1</sup> )	10.79 ± 1.2 <sup>a</sup>	20.10 ± 1.2 <sup>a</sup>	10.78 ± 1.3 <sup>a</sup>	10.85 ± 1.1 <sup>a</sup>	10.45 ± 1.3 <sup>a</sup>
Iron (mg·kg <sup>-1</sup> )	11.8 ± 1.1 <sup>a</sup>	45.0 ± 4.2 <sup>b</sup>	47.6 ± 3.2 <sup>b</sup>	47.2 ± 3.6 <sup>b</sup>	43.6 ± 4.8 <sup>b</sup>

Mean value with different superscript letters (a, b) in each column are significantly different for  $P < 0.01$

The mineral premix added was modified for iron content. For induction of iron deficiency, this element was removed and replaced with an equivalent amount of wheat starch. Iron in the control diet originated from Fe(II) gluconate, which was also added to the products with and without dried mushrooms in amounts adequately covering the allowance for animals.

At the completion of the feeding period on the last day of the experiment, the animals were killed and blood and internal organs described above were collected for biochemical tests. In both stages of the study the following indexes were determined: total iron binding capacity (TIBC), erythrocytes, haemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Fe concentration in the blood serum, as well as Fe content in the liver and kidneys.

Throughout the entire period of feeding the animals were kept separately in semi-metabolic cages in a room with a 12 h light/dark cycle and temperature of 21 °C ± 2 °C and relative humidity of 65%. Food consumption was recorded every day, and once a week the animals were weighed. Apart from the test diets, the animals received water *ad libitum*. Efficiency of nutrition was determined on the basis of body weight gains in animals after the consumption of 100 gram diet [18].

**Analysis of biochemical parameters and Fe contents in blood and serum of rats.** The amounts of iron in blood serum were measured by colorimetric method at wave length of 623 nm, while haemoglobin content was determined by

cyanmethemoglobin photometry using a 540 nm filter. Analyses were performed using a Vitalab Flexor apparatus (Vital Scientific NV, Dieren, The Netherlands). The degree of iron transferring saturation (TSAT) was calculated according to the following formula:  $TSAT = (Fe/TIBC) \times 100$ .

Values of morphological indexes were determined using a Sysmex K-1000 haematological analyser (TAO Medical Electronics Co., Kobe, Japan).

**Analysis of Fe contents in organs.** Analytical samples of organs (1 g) were wet mineralized with concentrated nitric acid (65% HNO<sub>3</sub> GR ISO) (Merck) in a MARS-5 microwave system (CEM Corp., Matthews, NC, USA). The obtained clear mineralizate was transferred to 25mL polypropylene flasks (PP) and made up to the mark with deionized water. Next, the mineralizate was diluted 5 or 10-fold with deionized water in the case of Fe assays from liver samples, while for analyses of Fe from kidney samples, the mineralizate was diluted 3-fold. Content of Fe was determined by flame atomic absorption spectrometry at a wavelength of 248.3 nm and gap width of 0.15 nm. Iron analyses were performed using an AAS-3 spectrometer (Carl-Zeiss, Analytik Jena AG, Jena, Germany). Accuracy and precision (99.6%) of the method were assessed based on the analysis of a certified reference material (pig kidney, BCR-186, Community Bureau of Reference, Brussels, Belgium).

**Statistical analysis of results.** All statistical calculations were performed using the StatSoft STATISTICA™ PL, version 9.0 programme. Basic descriptive statistics were calculated for the parameters. Means of analysed traits in the groups of animals, depending on the diet type, were compared using one-way analysis of variance, while intergroup differences were assessed by the LSD Fisher test. Intergroup differences at  $p < 0.01$  were marked between columns in the Tables using different letters.

## RESULTS

The aim of the first stage of the study was to induce iron deficiency in rats, which were used in experiments assessing iron bioavailability from products enriched with dried *Pleurotus ostreatus* mushrooms. The diets administered in the first stage of feeding contained reduced Fe levels in the mineral mix (Tab. 3). Lowered amount of this mineral significantly influenced parameters on iron metabolism indexes (Tab. 4). A significant drop was observed in haemoglobin by 17%, haematocrit – 11.5%, blood serum Fe content – 30.5%, Fe content in the liver – 38%, and Fe level in kidneys – 43%. Significant reductions were also recorded for MCV, MCH and MCHC values. In the group of animals fed the iron-deficit diet, a low percentage of iron transferrin saturation (TSAT) was also found.

No changes were observed in either of the groups of rats in general nutrition indexes (Tab. 4), counts of leukocytes and erythrocytes in the blood or weights of internal organs (liver, kidneys, spleen and heart).

In the second stage of the study rats, with the previously induced Fe deficiency were fed maize products enriched with dried *Pleurotus ostreatus* mushrooms in order to assess Fe bioavailability from these products. Table 5 presents general nutrition indexes, such as weights of internal organs and iron

**Table 4.** Nutritional indices, mass of internal organs and iron status in rats with induced anaemia in the first stage of the study (mean  $\pm$  SD)

Variable	Experimental group	
	Fe deficient diet	Control diet
Feed intake (g)	719 $\pm$ 20 <sup>a</sup>	721 $\pm$ 4.0 <sup>a</sup>
Initial body weight (g)	158 $\pm$ 14.5 <sup>a</sup>	165 $\pm$ 22 <sup>a</sup>
Body mass gain (g)	154 $\pm$ 75 <sup>a</sup>	147 $\pm$ 16.5 <sup>a</sup>
Feeding efficiency ratio (g·kg <sup>-1</sup> )	196 $\pm$ 21.9 <sup>a</sup>	187 $\pm$ 21.5 <sup>a</sup>
Dietary Fe intake (mg·kg <sup>-1</sup> )	8.51 $\pm$ 0.23 <sup>a</sup>	32.5 $\pm$ 0.18 <sup>b</sup>
WBC (G·L <sup>-1</sup> )	2.65 $\pm$ 0.99 <sup>a</sup>	3.05 $\pm$ 0.74 <sup>a</sup>
Liver (g)	9.49 $\pm$ 0.42 <sup>a</sup>	9.78 $\pm$ 1.00 <sup>a</sup>
Kidneys (g)	2.22 $\pm$ 0.24 <sup>a</sup>	2.11 $\pm$ 0.28 <sup>a</sup>
Spleen (g)	0.68 $\pm$ 0.13 <sup>a</sup>	0.61 $\pm$ 0.01 <sup>a</sup>
Heart (g)	1.03 $\pm$ 0.11 <sup>a</sup>	1.00 $\pm$ 0.05 <sup>a</sup>
TSAT	6.71 $\pm$ 1.50 <sup>a</sup>	20.7 $\pm$ 1.71 <sup>b</sup>
RBC (T·L <sup>-1</sup> )	7.97 $\pm$ 0.50 <sup>a</sup>	7.65 $\pm$ 0.48 <sup>a</sup>
Hb (mmol·L <sup>-1</sup> )	7.38 $\pm$ 0.46 <sup>a</sup>	8.87 $\pm$ 0.43 <sup>b</sup>
Ht (L·L <sup>-1</sup> )	0.38 $\pm$ 0.013 <sup>a</sup>	0.43 $\pm$ 0.009 <sup>b</sup>
MCV (fL)	47.3 $\pm$ 0.83 <sup>a</sup>	55.7 $\pm$ 0.91 <sup>b</sup>
MCH (fmol)	0.0924 $\pm$ 0.032 <sup>a</sup>	1.16 $\pm$ 0.024 <sup>b</sup>
MCHC (mmol·L <sup>-1</sup> )	19.5 $\pm$ 0.34 <sup>a</sup>	20.8 $\pm$ 0.16 <sup>b</sup>
Serum Fe (mmol·L <sup>-1</sup> )	12.28 $\pm$ 0.95 <sup>a</sup>	17.67 $\pm$ 1.22 <sup>b</sup>
Liver Fe ( $\mu$ g·g <sup>-1</sup> dry matter)	247 $\pm$ 24.1 <sup>a</sup>	399 $\pm$ 19.3 <sup>b</sup>
Kidney Fe ( $\mu$ g·g <sup>-1</sup> dry matter)	153 $\pm$ 13.5 <sup>a</sup>	269 $\pm$ 15.0 <sup>b</sup>

Mean value with different superscript letters (a, b) in each column are significantly different for  $P < 0.01$

metabolism indexes at the completion of the experiment consisting in feeding the animals with products in relation to the control groups, i.e. animals consuming an Fe-deficient diet and cereal products with no dried mushroom added. No changes were observed in general nutrition indexes in any group of animals. Weights of kidneys and the heart were similar in all male rats, whereas higher weights of the liver and the spleen were recorded in animals from the control group and the Fe-deficient group.

Feeding male Wistar rats for 26 days with an iron deficient diet and supplemented with dried *Pleurotus ostreatus* mushrooms resulted in the restitution of the systemic Fe level, as manifested by an increase of the level comparable to the control group for iron transferrin saturation rate, haemoglobin and MCV. It was found that MCH and MCHC values increased considerably, even compared to the control group. Values of haematocrit, MCH and MCHC in animals fed products supplemented with *Pleurotus ostreatus* were significantly higher compared to animals fed products with no Fe added. The highest MCV value was recorded when 20% of dried oyster mushrooms was added.

Iron levels in the blood serum, the liver and kidneys in animals fed cereal products considerably exceeded values recorded at the beginning of the experiment, and were similar to the control values. Contents of Fe in the liver and kidneys in animals from groups CB1 and CB2 were much higher than that in group C0.

In male rats administered the Fe-deficient diet a considerable deterioration of Fe deficit was observed, manifested in a reduction of haemoglobin content by 12.6%, MCV by 18%, serum Fe contents by 10%, Fe contents in kidneys by 15.7%, and a drop in Fe contents in the liver by 44%.

**Table 5.** Nutritional indices, internal organs masses, and status of iron in rats after feeding products with *Pleurotus ostreatus* in the second stage of the study (mean  $\pm$  SD)

Variable	Experimental group				
	CB1	CB2	CO	Deficit	Control
Feed intake (g)	493 $\pm$ 56.5 <sup>a</sup>	513 $\pm$ 38.2 <sup>a</sup>	443 $\pm$ 74.9 <sup>a</sup>	533 $\pm$ 19.5 <sup>a</sup>	551 $\pm$ 30.4 <sup>a</sup>
Initial body weight (g)	309 $\pm$ 16.9 <sup>a</sup>	309 $\pm$ 16.7 <sup>a</sup>	307 $\pm$ 7.21 <sup>a</sup>	311 $\pm$ 22.4 <sup>a</sup>	320 $\pm$ 30.6 <sup>a</sup>
Body mass gain (g)	44.4 $\pm$ 14.5 <sup>a</sup>	38.4 $\pm$ 10.0 <sup>a</sup>	23.3 $\pm$ 25.7 <sup>a</sup>	49.8 $\pm$ 9.44 <sup>a</sup>	48.2 $\pm$ 7.88 <sup>a</sup>
Feeding efficiency ratio (g·kg <sup>-1</sup> )	76.9 $\pm$ 18.7 <sup>a</sup>	65.3 $\pm$ 13.6 <sup>a</sup>	41.2 $\pm$ 43.7 <sup>a</sup>	81.6 $\pm$ 15.7 <sup>a</sup>	76.2 $\pm$ 14.0 <sup>a</sup>
Dietary Fe intake (mg·kg <sup>-1</sup> )	23.4 $\pm$ 2.68 <sup>b</sup>	24.2 $\pm$ 1.81 <sup>b</sup>	19.3 $\pm$ 3.27 <sup>b</sup>	6.31 $\pm$ 0.23 <sup>a</sup>	24.8 $\pm$ 1.37 <sup>b</sup>
WBC (G/l)	3.20 $\pm$ 0.87 <sup>a</sup>	3.06 $\pm$ 1.29 <sup>a</sup>	1.75 $\pm$ 0.35 <sup>a</sup>	3.33 $\pm$ 0.99 <sup>a</sup>	3.21 $\pm$ 0.91 <sup>a</sup>
Liver (g)	10.1 $\pm$ 1.13 <sup>b</sup>	8.85 $\pm$ 1.20 <sup>a</sup>	7.26 $\pm$ 0.14 <sup>a</sup>	9.37 $\pm$ 0.84 <sup>b</sup>	10.6 $\pm$ 0.90 <sup>b</sup>
Kidneys (g)	2.12 $\pm$ 0.12 <sup>a</sup>	2.18 $\pm$ 0.16 <sup>a</sup>	1.85 $\pm$ 0.28 <sup>a</sup>	2.24 $\pm$ 0.17 <sup>a</sup>	2.30 $\pm$ 0.29 <sup>a</sup>
Spleen (g)	0.49 $\pm$ 0.07 <sup>a</sup>	0.50 $\pm$ 0.08 <sup>a</sup>	0.42 $\pm$ 0.07 <sup>a</sup>	0.64 $\pm$ 0.02 <sup>b</sup>	0.63 $\pm$ 0.06 <sup>b</sup>
Heart (g)	0.99 $\pm$ 0.09 <sup>a</sup>	0.99 $\pm$ 0.09 <sup>a</sup>	0.84 $\pm$ 0.03 <sup>a</sup>	1.01 $\pm$ 0.12 <sup>a</sup>	1.00 $\pm$ 0.11 <sup>a</sup>
TSAT	21.6 $\pm$ 2.74 <sup>b</sup>	25.5 $\pm$ 7.22 <sup>b</sup>	22.3 $\pm$ 4.46 <sup>b</sup>	12.3 $\pm$ 1.11 <sup>a</sup>	22.5 $\pm$ 0.72 <sup>b</sup>
RBC (T·L <sup>-1</sup> )	5.79 $\pm$ 1.73 <sup>a</sup>	5.06 $\pm$ 2.35 <sup>a</sup>	6.53 $\pm$ 0.14 <sup>a</sup>	9.12 $\pm$ 1.24 <sup>a</sup>	8.78 $\pm$ 1.23 <sup>a</sup>
Hb (mmol·L <sup>-1</sup> )	8.19 $\pm$ 1.60 <sup>b</sup>	8.44 $\pm$ 1.08 <sup>b</sup>	7.88 $\pm$ 0.95 <sup>b</sup>	6.45 $\pm$ 1.07 <sup>a</sup>	9.55 $\pm$ 1.43 <sup>b</sup>
Ht (l·L <sup>-1</sup> )	0.362 $\pm$ 0.015 <sup>b</sup>	0.37 $\pm$ 0.01 <sup>b</sup>	0.34 $\pm$ 0.01 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>a</sup>	0.46 $\pm$ 0.03 <sup>c</sup>
MCV (fL)	51.4 $\pm$ 1.98 <sup>b</sup>	56.6 $\pm$ 1.36 <sup>c</sup>	53.0 $\pm$ 1.39 <sup>b</sup>	38.8 $\pm$ 0.83 <sup>a</sup>	52.9 $\pm$ 1.82 <sup>c</sup>
MCH (fmol)	1.61 $\pm$ 0.18 <sup>c</sup>	1.51 $\pm$ 0.15 <sup>c</sup>	1.21 $\pm$ 0.11 <sup>b</sup>	0.97 $\pm$ 0.08 <sup>a</sup>	1.09 $\pm$ 0.03 <sup>b</sup>
MCHC (mmol·L <sup>-1</sup> )	30.8 $\pm$ 1.65 <sup>b</sup>	26.8 $\pm$ 1.98 <sup>b</sup>	22.7 $\pm$ 1.88 <sup>a</sup>	25.1 $\pm$ 2.00 <sup>a</sup>	20.6 $\pm$ 1.47 <sup>a</sup>
Serum Fe (mmol·L <sup>-1</sup> )	18.6 $\pm$ 0.96 <sup>b</sup>	18.6 $\pm$ 2.18 <sup>b</sup>	18.09 $\pm$ 2.90 <sup>b</sup>	11.05 $\pm$ 2.33 <sup>a</sup>	19.5 $\pm$ 4.33 <sup>b</sup>
Liver Fe ( $\mu$ g·g <sup>-1</sup> dm)	349 $\pm$ 15.8 <sup>c</sup>	384 $\pm$ 15.4 <sup>c</sup>	322 $\pm$ 11.6 <sup>b</sup>	138 $\pm$ 21.6 <sup>a</sup>	372 $\pm$ 29.6 <sup>c</sup>
Kidney Fe ( $\mu$ g·g <sup>-1</sup> dm)	265 $\pm$ 21.4 <sup>c</sup>	262 $\pm$ 20.8 <sup>c</sup>	208 $\pm$ 18.5 <sup>b</sup>	129 $\pm$ 32.2 <sup>a</sup>	212 $\pm$ 34.8 <sup>bc</sup>

Mean value with different superscript letters (a, b) in each column are significantly different for  $P < 0.01$

Based on the obtained results, a restitution was observed within a relatively short time of earlier iron deficiency in male rats. Consumption of maize products enriched with dried *Pleurotus ostreatus* increased iron contents in kidneys and the liver, and resulted in an increase in MCV, MCH and MCHC values.

## DISCUSSION

In the opinions of many researchers [9, 11, 12, 13, 14, 15, 16] oyster mushroom *Pleurotus ostreatus* is a good source of soluble and insoluble dietary fibre, vitamins B (including B<sub>12</sub>), as well as micro- and macro-elements, such as K, Mg, P, Zn

and Fe. Iron content in fresh oyster mushroom, on average, is 14.3 mg/100g. While there is an abundance of information on the nutritive value of oyster mushroom in literature, there is a very limited number of studies concerning cereal products enriched with this raw material and the bioavailability of minerals from such products. Bioavailability, according to Fairweather-Tait [19], is the degree to which a consumed component, following its release from bonds found in food, may be absorbed in the alimentary tract and utilised by the organism. Studies on bioavailability and digestibility of nutrients are not very well developed. In the preparation of formulas for novel food products it is essential to determine to what degree nutrients contained in the foodstuff may be utilised by the organism [20]. Different experimental methods are used in the assessment of iron bioavailability from the diet or a supplement, such as, e.g. methods using isotopes (radioactive and stable isotopes) assessing the degree of absorption of these markers or models with animals with induced deficiencies of this nutrient, and evaluating the rate of restitution for these deficits (e.g. repletion/slope ratio method, haemoglobin regeneration assay). In this case, after an induced iron deficit, animals are administered a diet containing the tested iron compound, and the degree of its utilisation is assessed on the basis of the levels of bioactive forms (Hb, ferritin, Fe reserves in liver), in comparison to the reference standard iron form (e.g. FeSO<sub>4</sub>) [21]. Such a model was applied in the conducted experiment, in which Wistar rats were fed a deficit diet, devoid of an iron addition in the mix of minerals (approx. 25% recommended amount). Following a diagnosed iron deficit in the organism the animals were fed with products supplemented with dried oyster mushroom in order to determine Fe bioavailability from these products in comparison to the complete control diet and the deficit diet.

In the first stage of the analyses, iron deficit was successfully induced in the organisms of the animals, which resulted in a reduction of morphological blood parameters as well as Fe level in blood serum, the liver and kidneys of the male rats. Collected data were similar to the results reported by the authors in another experiment using model animals with Fe deficit [22]. Such an animal model was successfully established within a relatively short time. According to Clark [5], iron metabolism is highly efficient, as Fe released from decomposing erythrocytes returns to the plasma, or is stored. At an insufficient consumption of iron, first, a negative balance is observed, together with the depletion of reserves, followed by biochemical changes, reflecting the deficit of iron required for the production of haemoglobin. Only at a longer-lasting deficit, anaemia caused by iron deficiency starts to develop.

In the second stage of the study, rats with iron deficit were fed with products supplemented with a 10% or 20% dried oyster mushroom addition. Such a procedure caused a gradual release of Fe reserves, reduced in the earlier experiment. This was indicated by the significant changes in Fe metabolism indexes, such as an increase to the level comparable to that in the control for iron transferrin saturation rate, increased concentrations of haemoglobin, haematocrit, MCV, MCH and MCHC. Iron concentration in blood serum and the content of this nutrient in the liver and kidneys also increased considerably. At the same time, it needs to be stressed that already enrichment of the product with a 10% addition of dried oyster mushroom caused a significant restoration of

systemic iron level in those animals. Suliburska [23, 24] expressed an opinion that products enriched with Fe are better sources of potentially available Fe than products not enriched with this nutrient, but the sorption of minerals depended on the dietary fibre source, adsorbent type, processing method, pH and temperature of the medium, as well as on the metal. Regula and Gramza-Michalowska [25] observed in *in vitro* experiments, maximum sorption values at pH = 8.7 for Fe in bread with 10% and 20% dried mushrooms added (15% and 17%, respectively). The sorption of Fe at pH 1.8 did not exceed 3%.

## CONCLUSIONS

Feeding male rats with products supplemented with dried oyster mushroom *Pleurotus ostreatus* caused a restitution of reduced iron reserves within a relatively short time. Bioavailability of iron from enriched products was high and comparable to the bioavailability of this nutrient from the reference standard form, i.e. Fe(II) gluconate. Production of cereal snacks naturally enriched with iron of good bioavailability may extend the assortment of products used in nutrition prevention practice. Such a product may be a valuable source of iron in the nutrition of individuals with a deficiency of this element, primarily patients with absorption and metabolism disorders, but also may add variety to a traditional daily diet.

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