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DETECTION OF SELECTED HEAVY METALS AND MICRONUTRIENTS IN EDIBLE INSECT AND THEIR DEPENDENCY ON THE FEED USING XRF SPECTROMETRY

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ABSTRACT

Edible insect can be a valuable source of nutrients, but also a potential source of heavy metals. Quick detection of over-limit heavy metals concentration could be a key to processing and quick distribution of edible insect products. The aim of this work was to evaluate the feed-dependent content of heavy metals in the mealworm and superworm using the X-ray fluorescence spectrometry as an easy, cheap and a timeless screening method for evaluating the content of heavy metals and microelements. Using a handheld analyser the content of Cd, Pb, Cu and Zn were detected. Both analysed species proved dependency of metal content on a feed. Detected level of Cu in mealworm was between 571 $mg.kg^{-1}$ and 1768 $mg.kg^{-1}$ and in superworm from 571 $mg.kg^{-1}$ to 1768 $mg.kg^{-1}$ based on the feed. The content of Zn was similar, between 725 $mg.kg^{-1}$ and 1437 $mg.kg^{-1}$ in mealworm and 555-1482 $mg.kg^{-1}$ in superworm. The level of Pb was below the detection limit in all samples, thus from this point of view this food seems to be safe. On the contrary, the content of Cd in the dry matter samples was above the food limit – 147 $mg.kg^{-1}$ to 230 $mg.kg^{-1}$. From this point of view, the samples were evaluated as unsuitable for consuming.

Keywords: edible insect; mealworm; superworm; X-ray fluorescence spectrometry; heavy metal

INTRODUCTION

Edible insect is an important food source for more than 2 billion people, especially in developing countries. Given the ever-growing cost of animal protein production and the ecological consequences of livestock farming, edible insect appears to be a very important strategic resource **(Mlček et al., 2014)**, because the demand for animal commodities such as beef, pork and fish meat is constantly increasing **(van Huis, 2013; Belluco et al., 2013; Menzel and D'Aluisio, 1998; DeFoliart, 2002; Paoletti, 2005)**. Although in developed western countries the entomophagy is often associated with disgust, primitive behaviour, poverty and diseases; edible insect is also becoming an interesting commodity **(De Foliart, 1992; Ramos-Elorduy et al., 2006)**. The demand for products made of edible insects is gradually increasing even in European countries, but until now the consumption of edible insects has not been spread to a greater extent. Thus, according to the **Regulation (EU)** of the European Parliament and of the **Council 2015/2283** on novel foods, edible insect from the 1st January 2018 belongs to the novel foods in the countries of the EU.

The insect is highly nutritive and healthy source of food with higher content of fat, protein, vitamins, fiber and minerals **(van Huis, 2013)**. However, insect can accumulate dangerous chemicals, including heavy metals, in its tissues **(Handley et al., 2007; Zhuang et al., 2009)**, along with dioxins **(Devkota and Schmidt, 2000)** and flame retardants **(Gaylor et al., 2012)**. Risks connected with the consumption of edible insects were also addressed by EFSA in the document „Risk profile related to production and consumption of insects as food and feed“ published in October 2015 **(EFSA, 2015)**.

Cd, Pb, Zn a Cu belong to the highly toxic and relatively accessible elements **(Toman, 2003; Pavlovský, 2014)**. The impact of cadmium exposure on the human organism is wide - from gastroenteritis and the possibility of osteomalacia to carcinogenic and teratogenic effects **(Velíšek, 2002; Toman, 2005)**. WHO suggests a maximum weekly intake of cadmium 7000 $mg.kg^{-1}$. High content of cadmium can be found in wheat, rice, mussels and animal kidney corns **(Oymak et al., 2009)**. The limit for cadmium content in the meat of cattle, sheep, pigs and poultry is 0.05 $mg.kg^{-1}$ fresh weight, crustaceans 0.50 $mg.kg^{-1}$ fresh weight, clams 1.00 $mg.kg^{-1}$ fresh weight and cephalopods 1.00 $mg.kg^{-1}$ fresh weight **(Commission Regulation (EC) No 1881/2006)**.

A typical toxic element, which has no physiological function in the body, is lead. The effect of lead on the human organism is, as with cadmium, very wide – from gastroenteritis to neurotoxic effects **(Velíšek, 2002; Sola et al., 1998)**. Children have experienced decreased intelligence and anemia when exposed to very low doses **(Memon et al., 2005)**. Limit for lead content in the meat of cattle, sheep, pigs and poultry is 0.10 $mg.kg^{-1}$ fresh weight, crustaceans 0.50 $mg.kg^{-1}$ fresh weight, clams 1.50 $mg.kg^{-1}$ fresh weight and cephalopods 1.00 $mg.kg^{-1}$ fresh weight **(Commission Regulation (EC) No 1881/2006)**.

Zinc is an essential element that is part of enzymes, but at higher concentrations it is toxic. Zinc ingestion causes gastrointestinal problems, such as osteoporosis, in case of long-term exposure. Higher doses result in disorders of cholesterol metabolism, resulting in atherosclerosis **(Toman, 2003; Pavlovský, 2014)**. Recommended daily intake, according to **Decree No. 352/2009 Coll. (CZ)** is 10 mg.

Copper is essential for life, is part of enzymes and important for hematopoiesis. Exposure tolerance in adults is high, but children are poisoned at low concentrations **(Toman, 2003; Pavlovský, 2014)**. Recommended daily intake, according to **Decree No. 352/2009 Coll. (CZ)** is 1 mg.

Due to an insufficient examination of edible insect safety as a novel food for humans in the EU, it is necessary to analyse not only the nutritional value of farmed insects, but also to know the influence of insect consumption on the human health, its risks and safety. Given the uncertainties in European law, it is not yet clear what regulation and maximum levels of contaminants are to be applied to edible insects as a novel food. Before introducing insects to the European market, it is therefore necessary to amend and clarify the legislation and along with it the maximum levels of contaminants **(Spiegel, 2013)** including heavy metals **(EFSA, 2015)**. At present, however, the toxicological limits of heavy metals in insect insects are not legislatively established, and therefore limits for the crustaceans are used, as the crustaceans are anatomically related to edible insects and have similar allergens (chitin).

Hyun (2012), **Zielinska (2015)**, **Nowak (2016) and Poma (2017)** published chemical analyses with heavy metal contents in different insect species. It is assumed that the dependency of the content of selected elements in the body of insects depends mainly on the feed, the species and the breeding environment. E.g. **Oonincx (2011)** states that in Locusta migratoria, the change of feed led to changes in the content of copper from 24.5 to 28.5 $mg.kg^{-1}$ in penultimate instars and from 33.8 to 41.3 $mg.kg^{-1}$ in adults. In his study, the content of zinc detected was 137-150 $mg.kg^{-1}$ in penultimate instars and 137 – 172 $mg.kg^{-1}$ in adults. However, these studies do not focuse either on edible insects kept in Central European conditions or on the rapid determination of metallic content in edible insects using X-ray spectrometry methods.

The aim of this study was to determine the selected elements' content using the X-ray fluorescence spectrometry in mealworm (*Tenebrio molitor*) and superworm (*Zophobas morio*) bred in the Czech Republic and to evaluate the influence of feed on the content of heavy metals and nutrients.

Scientific hypothesis

Scientific hypothesis is: The content of metals in the edible insect varies according to the type of feed and this change can be measured by means of a handheld X-ray spectrometer.

MATERIAL AND METHODOLOGY

Material

Chemicals

There were used:

- HNO_3 p. a., Mr. 63.01, Penta, Praha, CZ,
- H_2O_2 p. a., Mr. 34.02, Penta, Praha, CZ,
- $CdCl_2$ p. a., Mr. 183.32, CAS No.: [10108-64-2], Fluka analytical, Sigma Aldrich,
- $ZnCl_2$ p. a., Mr. 136.29, ML chemical, Troubsko, CZ,
- $PbCl_2$ p. a., Mr. 278.11, Lachema, n. p., Brno, CZ,
- $CuCl_2 .2\,H_2O$ p. a., Mr. 170.48, CAS No.: [10125-13-01], ML chemical, Troubsko, CZ,
- CH_3COOH p. a., Mr. 60.05, CAS No.: [64-19-7],
- Deionized water, 18.2 MOhm.cm, Milli-Q, Millipore.

All chemicals were of analytical reagent grade or equivalent analytical purity.

Insect

Larvae samples of the following species were used for analyses: superworm (*Zophobas morio*), mealworm (*Tenebrio mollitor*). Samples were purchased at the Hostivice Feed Shop. The insects were kept in optimal conditions for development of each species. Insect species were divided into three experimental groups. The first group was fed with wheat bran, the second group was fed with oat bran, and the third group was fed with soy flour. All groups were fed ad libitum. Before the analysis, all insect samples of all species were modified as follows: larvae in the last and penultimate growth stages (full length of the body just prior to pupation) were taken. The next steps were: starving for 48 hours, killing with boiling water (100 °C) and drying at 105 °C. The samples prepared were homogenized and stored in a refrigerated box at 4 – 7 °C until analysis. In the next step, feed was analysed - wheat bran, oat bran and soy flour.

Nutrition values of the feed

Data by manufacturer are per 100 g of product:

- Wheat bran/crude: energy value: 1,210 kJ / 292kcal, fats 5.3 g, of which saturated fatty acids 0.88 g, carbohydrates 24.9 g, of which sugars 2.2 g, fiber 40.2 g, protein 16.2 g, salt 0.1 g. Company: Country Life, s.r.o., Beroun 1.
- Oat bran: energy value: 73.95 kJ / 17.67 kcal, fats 0.390 g, of which saturated fatty acids 0.070 g, carbohydrates 2.675 g, proteins 0.825 g, fiber 0.730 g, salt 0.004 g. Company: Natural Jihlava JK s.r.o., Jihlava.
- Soy flour/crude, whole: energy value: 1.770 kJ / 423 kcal, fats 20.7 g, of which saturated fatty acids 3 g, carbohydrates 25.6 g, of which sugars 7.5 g, protein 34.5 g, salt 0 g. Company: Paleta s.r.o., Lipnice 152.

Analyses of selected elements

A homogeneous 0.1 g of sample was placed in a tube, followed by the addition of 2 mL of 65% HNO_3. The metals were extracted for 24 hours at room temperature and then heated to 110 °C for 1 hour. Next, 200 µL of 30% H_2O_2 was added and the sample was heated for a further 30 minutes. After cooling, the sample was diluted with 5 times deionized water (v/v) (18.2 MOhm.cm, Milli-Q, Millipore).

In XRF spectrometry, the sample is identified by its radiation emission of a characteristic wavelength or energy. The amount of elements present is determined by measuring the intensity of its characteristic wave energy. The handheld ED-XRF spectrometer Innov-X DELTA (Innov-X Systems, INC., Woburn, USA) was used for the measurement. Samples were put in a special measuring capsule and then placed in a measuring box for analysis. The analysis was started using the control program DELTA Premium PC Software (Innov-X Systems, INC.).

Statisic analysis

The data were analysed using Excel 2013 (Microsoft Corporation, USA) and STATISTICA CZ version 12 (StatSoft, Inc., USA). The data obtained from experiment were evaluated according to basic statistical characteristic and results were expressed by average ±standard deviation.

Since the measured values of spikes and obliquities correspond to the normal distribution, the parametric statistical test ANOVA was used.

Comparison of the results was performed using a Fisher LSD assay ($\alpha = 0.05$).

RESULTS

In this study, in the first place the commodities used for feeding were analysed. In the next stage the insect itself was analysed. From the feed analysis results, it is clear that the largest amount of metals is found in wheat bran. In addition to the monitored elements, iron was also detected. Lead was below the detection limit in all the measured samples. Similarly, copper was also often below the detection threshold for oat bran and soy flour. The statistical values of the monitored elements in the feed are shown in Table 1.

Next, element content was measured in samples of insects (mealworm and superworm) fed with various feeds (wheat bran, oat bran and soy flour). The mean and standard deviation of the monitored elements (Cu, Zn, Cd and Pb) are shown in Table 2.

Each measurement was performed 3 times and statistically processed by the ANOVA method, also the Fisher LSD test was performed. Comparison of zinc, cadmium and copper content in superworm and mealworm using the Fisher LSD assay between groups of different feeds (wheat bran, oat bran and soybean meal) is shown in Table 3. This test indicates statistically significant differences in the content of the monitored metals in the edible insect depending on the feed.

In the case of copper and superworm, a statistically significant difference was found between the group fed with wheat bran fried and other groups fed with oat bran and soy flour. There was no statistically significant difference between the groups fed with oat bran and soy flour, but the result was close to its limit (p = 0.06). A statistically significant difference between the group fed with wheat bran and the other groups is shown again in the mealworm. Similarly, the group fed with oat bran is statistically different from all other groups.

For zinc, statistically significant differences between all monitored groups were found in both species. The exception were the groups of superworm fed with oat bran and soy flour, among which no statistically significant difference were confirmed. Furthermore, a statistically significant difference was found between the two species fed with the same feed (wheat bran, soy flour), but in the case of oat bran no statistically significant difference was detected.

Table 1 Content of metals and microelements in feed.

Feed	Content of metals			
	Cu ($mg.kg^{-1}$ ±SD)	**Zn ($mg.kg^{-1}$ ±SD)**	**Cd ($mg.kg^{-1}$ ±SD)**	**Pb ($mg.kg^{-1}$ ±SD)**
Wheat bran	586 ±43	1095 ±72	102 ±11	<LOD
Oat bran	293 ±38	322 ±46	72 ±6	<LOD
Soy flour	315 ±16	415 ±25	74 ±13	<LOD

Table 2 Concentrations of metals and microelements in analysed samples of mealworm (TM) and superworm (ZM) with different feed (wheat bran, oat bran and soy flour).

Species	Feed	Content of metals			
		Cu ($mg.kg^{-1}$ ±SD)	**Zn ($mg.kg^{-1}$ ±SD)**	**Cd* ($mg.kg^{-1}$ ±SD)**	**Pb ($mg.kg^{-1}$ ±SD)**
ZM	Wheat bran	1768 ±131	1482 ±43	**230 ±9**	<LOD
ZM	Oat bran	828 ±138	666 ±21	**163 ±22**	<LOD
ZM	Soy flour	571 ±34	555 ±71	**147 ±28**	<LOD
TM	Wheat bran	1201 ±108	1071 ±24	**183 ±23**	<LOD
TM	Oat bran	767 ±56	725 ±92	**157 ±18**	<LOD
TM	Soy flour	1866 ±293	1437 ±143	**186 ±27**	<LOD

Note: * The Cd value exceeds the limit of 1.00 $mg.kg^{-1}$ in fresh weight, (Commission Regulation (EC) No 1881/2006).

In the case of cadmium, a statistically significant difference between the group of superworm fed with wheat bran and superworm groups fed with other feed was confirmed. There was no statistically significant difference between the other monitored groups.

DISCUSSION

In this study, two basic heavy metals (Cd, Pb), which are highly toxic to humans, and two microelements (Cu, Zn) have been investigated. These two microelements are important from the health point of view and their intake needs to be properly balanced to avoid undesirable health problems **(The Czech Society for Nutrition, 2011)**.

In the case of superworm (*Zophobas morio* – ZM), the content of the monitored elements (except for the lead, which was below the detection limit), had a similar trend as for the feed that the insect was fed with. The high content of metals in wheat was reflected in the total content of the monitored metals in the body of the superworm. For other feeds with the lower content of the monitored elements, the content of these elements was also reduced in the body of the superworm.

For the mealworm (*Tenebrio molitor* – TM), the content of the observed elements in the insect body was higher when fed with wheat bran than oat bran. However, when using soy flour as feed, the observed microelements and Cd in the body of insects increased significantly in comparison with bran.

The copper content was between 571 – 1,768 $mg.kg^{-1}$ in superworm, and 767 $mg.kg^{-1}$ to 1,866 $mg.kg^{-1}$ in mealworm. These values indicate a high concentration of copper in these species, so they can serve as the source of this element. Compared with other authors, its content is significantly higher. A similar trend was also found in zinc, ranging from 555 $mg.kg^{-1}$ to 1482 $mg.kg^{-1}$ in superworm, and 725 $mg.kg^{-1}$ to 1437 $mg.kg^{-1}$ in mealworm.

The average cadmium content was higher than 147 $mg.kg^{-1}$ in all monitored samples and significantly exceeds the limit of 1.00 $mg.kg^{-1}$ in the fresh weight. For this reason, all analysed samples are potentially dangerous. The lead content was below the limit of detection for both species analysed and for all feeds used. **Poma (2017)**, who studied nine minerals in his study, also came to this

Table 3 Comparison of zinc, copper and cadmium content in the analysed samples of mealworm (TM) and superworm (ZM) depending on feed (wheat bran, oat bran and soy flour) using the Fisher LSD test.

Species	Feed	Content of copper					
		ZM Wheat bran	ZM Oat bran	ZM Soy flour	TM Wheat bran	TM Oat bran	TM Soy flour
ZM	Wheat bran		0.00	0.00	0.00	0.00	0.45
ZM	Oat bran	0.00		0.06	0.00	0.64	0.00
ZM	Soy flour	0.00	0.06		0.00	0.15	0.00
TM	Wheat bran	0.00	0.00	0.00		0.00	0.00
TM	Oat bran	0.00	0.64	0.15	0.00		0.00
TM	Soy flour	0.45	0.00	0.00	0.00	0.00	

Species	Feed	Content of zinc					
		ZM Wheat bran	ZM Oat bran	ZM Soy flour	TM Wheat bran	TM Oat bran	TM Soy flour
ZM	Wheat bran		0.00	0.00	0.00	0.00	0.52
ZM	Oat bran	0.00		0.13	0.00	0.41	0.00
ZM	Soy flour	0.00	0.13		0.00	0.03	0.00
TM	Wheat bran	0.00	0.00	0.00		0.00	0.00
TM	Oat bran	0.00	0.41	0.03	0.00		0.00
TM	Soy flour	0.52	0.00	0.00	0.00	0.00	

Species	Feed	Content of cadmium					
		ZM Wheat bran	ZM Oat bran	ZM Soy flour	TM Wheat bran	TM Oat bran	TM Soy flour
ZM	Wheat bran		0.00	0.01	0.00	0.00	0.04
ZM	Oat bran	0.00		0.57	0.86	0.76	0.26
ZM	Soy flour	0.01	0.57		0.48	0.72	0.18
TM	Wheat bran	0.00	0.86	0.48		0.61	0.28
TM	Oat bran	0.00	0.76	0.72	0.61		0.16
TM	Soy flour	0.04	0.26	0.18	0.28	0.16	

conclusion. In the case of the mealworm, the Cu content was 5.81 mg.kg^{-1}, Zn 58.60 mg.kg^{-1}, Cd 0.06 mg.kg^{-1}, and the Pb content was lower than the detection limit. **Poma (2017)** proves in the study that insects can be a valuable source of micronutrients (Cu and Zn). The level of heavy metals detected in his study was lower than that of **Commission Regulation 1881/2006** and lower or comparable to conventional foods of animal origin.

Zielinska et al. (2015) also focused on the determination of minerals in edible insects, level of Cu for the Tenebrio molitor was 18.6 mg.kg^{-1} and for Zn 112 mg.kg^{-1} in their experiment. Recommended daily doses for humans reported by **Zielinská et al. (2015)** are 0.9 – 1.3 mg.day^{-1} for Cu and 3 – 14 mg.day^{-1} for Zn. ** (Linus Pauling Institute's Micronutrient Center). **Bukkens (2005)** states that insect is generally rich in minerals and their content is higher than that of the slaughtered animals. For mealworm the zinc content is comparable to beef (125 mg.kg^{-1} beef and 112 mg.kg^{-1} mealworm). **Finke (2004)**, in its review, reported a quantity of Cu for a mealworm 16 mg.kg^{-1} and Zn 137 mg.kg^{-1} and for superworm Cu 9 mg.kg^{-1} and Zn 73 mg.kg^{-1}. In 2015, **Finke (2004)** measured copper Cu (8.3 mg.kg^{-1}) and Zn (49.5 mg.kg^{-1}) and in superworm Cu 3.6 mg.kg^{-1} and Zn 30. 2 mg.kg^{-1}.

Analytical methods, which are often destructive and time-consuming for the preparation of samples, and in which concentrated acids are often used, were also used in the cited articles to analyse these values. Sample preparation for chemical analysis of elements usually takes from 20 minutes to several hours. Subsequent analysis is also time-consuming. The advantage of XRF spectrometry that was used in this work is its speed and simplicity of identification and quantification of basic elements over a wide range of concentrations ranging from several mg.kg^{-1} to practically 100% of weight. The sample is not destroyed by XRF spectrometry itself and its preparation is not time-consuming. The sample returns to its original state in milliseconds.

CONCLUSION

This study was the first step in finding a quick and simple method for determining the heavy metal content (Cd and Pb) and micronutrients (Cu and Zn) by XRF analysis in order to obtain safe food from selected insect species (mealworm, superworm). The results prove that the content of the monitored elements depends on the species and the feed. The lead level was below the detection limit for all species observed, thus the food appears to be safe from this point of view. On the contrary, higher cadmium, zinc and copper contents were found in the study. The cadmium content in samples exceed the allowed sanitary limits, therefore they are unsuitable for consumption. X-ray fluorescence spectrometry can serve as a primary screening for the detection of heavy metals and micronutrients in food commodities including edible insects. It is quick, simple, and financially undemanding method.

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