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NUTRITIONAL VALUES OF EDIBLE *COLEOPTERA* (*TENEBRIO MOLITOR*, *ZOPHOBAS MORIO* AND *ALPHITOBIUS DIAPERINUS)* REARED IN THE CZECH REPUBLIC

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ABSTRACT

Edible insects have gained the status of highly nutritious food with high protein and fat content. However, nutritional value of insects is not constant. It could be affected by species, developmental stage, rearing technology, nutrition or sex. This study's goal is to determine the protein and fat contents of three edible beetle species (giant mealworm – larvae of *Zophobas morio*, mealworm – larvae of *Tenebrio molitor* and, lesser mealworm – larvae of *Alphitobius diaperinus*) bred in the Czech Republic. Based on the obtained results, all investigated species could be considered as a reasonable source of lipids and two of them (mealworm and lesser mealworm) are also an excellent source of protein. Crude protein content of mealworm (630 g. kg^{-1} DM) was found to be higher than in other studies. The investigated species of lesser mealworm contained 600 g of crude protein/kg DM, which was equal to the results of other authors. Most authors report a higher content of nitrogen in the giant mealworm than were the values measured by this experiment (390 g.kg^{-1} DM). The lipid content in the tested samples was found in a range of 170 – 390 g.kg^{-1} DM. The highest lipid content was found in the larvae of giant mealworm and the lowest lipid content was found in the larvae of mealworm. The determined fat content of lesser mealworms was 290 g.kg^{-1}. The fatty acid profiles of all samples were also determined.

Keywords: edible insects; nutrition; protein, fat; fatty acid; *Coleoptera*

INTRODUCTION

Edible insects form a common part of the human diet in many parts of the world **(van Huis et al., 2013; Vantomme et al., 2012)**. They are also being considered an extra food source in countries where people have limited access to sufficient, safe and nutritious food to maintain a healthy and active life **(Kampmeier and Irwin, 2009; van Huis et al., 2013; Vantomme et al., 2012)**.

Edible insects are seen as an interesting alternative source of proteins and lipids **(Zielińska et al., 2015)**. They are also believed to be an ideal option for the space agriculture **(Katayama et al., 2008)**. In the developing countries, edible insects may serve as a potential animal protein source because of its better digestibility and utilization than vegetable protein **(Hoffman and Falvo, 2004)**. They could also help the children suffering from malnutrition **(Brázdová, 2011)**.

Some species of insects could serve as an important source of lipids. Fatty profile of insects varies among different species as well as among the developmental stages within one species **(Finke, 2004)**. It may also be easily affected by the feed composition **(Schaefer, 1968; Bukkens, 1997; Mariod, Abdel-Whab and Ain, 2011).** Fatty acid composition of insect is reported to be similar to that of poultry or fish **(DeFoliart, 1992)**.

Entomophagy is not very common in Europe. Insects are usually considered a delicacy or a means to diversify one's diet. Although the amount of information about the insects' nutritional composition and the potential risks has been recently increased, insects are still not considered a standard human food. A list of edible insects (including mealworm, giant mealworm, and lesser mealworm) was published by **EFSA (2015)** together with the risk related to production and consumption of insects as food and feed. Although entomophagy is considered to be safe due to its long history, manipulation with and consumption of edible insect may involve some risks **(EFSA, 2015)**. These risks are usually represented by collecting the insects in dangerous areas without protective equipment, consuming inappropriate developmental stages or inadequate culinary treatment **(Ramos-Elorduy, 2005; Belluco et al., 2013; Mlček et al., 2014)**. The toxic substances content or allergic reactions (mostly to chitin) are among other potential risks of edible insect consumption **(Park, Kim and Yang, 2009)**.

Available data about nutritional values of insect species bred in Europe are not sufficient. The mealworm is probably the most-studied species **(Bernard, Allen and Ullrey, 1997; Oonincx and Dierenfeld, 2012; Bednářová et al., 2013** and **van Broekhoven et al., 2015)**. It could be considered as a good source of protein and lipids, although the nutritional composition varies among individual developmental stages. The highest protein content (637.0 – 676.5 g.kg^{-1} DM) and the lowest fat content (148.8 – 184.0 g.kg^{-1} DM) were found in adult beetles. However, adult beetles are not very suitable for human consumption because of the high anti-nutritional substances content (wings, exoskeleton, legs etc.).

From the nutritional point of view the larvae (protein: 477.6 – 527.0 .kg^{-1} DM, fat 189.0 – 382.9 .kg^{-1} DM) and pupae (protein: 531.3 – 546.0 .kg^{-1} DM, fat

308.0 –366.5 g.kg^{-1} DM) seem to be more interesting. The nutrient content of hormonally modified mealworm form ("super mealworm") is known as well. These mealworms with artificially delayed pupation have the protein content comparable to other mealworms (471.8 g.kg^{-1} DM) but the fat content differs significantly (430.8 g.kg^{-1} DM) (calculated from **Finke, 2002**).

Giant mealworm, whose larvae could reach 55 mm **(Friedrich and Volland, 2004)**, is also considered to be a good source of quality protein and lipids. The nutritional composition of this species was determined by various authors **(Barker, Fitzpatrick and Dierenfeld, 1998; Finke, 2002; Bednářová et al., 2013; Yi et al., 2013; Bosch et al., 2014; van Broekhoven et al., 2015)**. The protein content of giant mealworm larvae was 431.3 – 516.2 g.kg^{-1} DM, the fat content was 328.0 – 435.4 g.kg^{-1} DM. **Oonincx and Dierenfeld (2012)** evaluated the nutrient content of giant mealworms adults and determined the protein level to be 680.5 g.kg^{-1} DM and lipid content 142.5 g.kg^{-1} DM.

The nutrient content of lesser mealworm is only available for larvae stages. **Bosch et al. (2014)** reported 648 g.kg^{-1} DM of protein and 222 g.kg^{-1} DM of fat. **Yi et al. (2013)** determined 580.3 g.kg^{-1} DM of protein and 239.5 g.kg^{-1} DM of fat. **Van Broekhoven et al. (2015)** found protein content to be 617 – 650 g.kg^{-1} DM and fat content 134 – 243 g.kg^{-1} DM.

Besides the factor of the above-mentioned development stage, the nutrient content of insects is also affected by feed composition, microclimate, environment, sex and other factors **(Oonincx and van der Poel, 2011)**. **Van Broekhoven et al. (2015)** reported that the feeding mixture change caused differences in content of both fat and protein (by 8 % and 11 % respectively). This research is therefore focused on the determination of basic nutrient contents of three edible insect species reared under defined farming conditions in the Czech Republic and the comparison of the obtained data with results from other countries and wild species.

MATERIAL AND METHODOLOGY

Material

The insect samples tested for the purposes of this study were larvae of darkling beetles (*Zophobas morio,* Fabricius, 1776), which are known by the common name superworm or giant mealworm, mealworm (larvae of *Tenebrio molitor*, Linnaeus, 1758) and lesser mealworm (larvae of *Alphitobius diaperinus*, Panzer, 1797). All of them are common warehouse pests and can be easily kept and bred in the European climate conditions. The samples were purchased in the ultimate or penultimate instars (most suitable to culinary purposes) from a private company Radek Frýželka, Brno. The insect species were fed by a mixture of plant material (carrots, cabbage, Chinese cabbage, tomatoes, and potatoes). Prior to the analysis, the insects were fasted for 48 hours to minimize the effects of food retained in the gut, then killed in boiling water (100 °C) and finally dried at 105 °C for 12 h. The obtained samples were then homogenized for 1 minute by the coffee grinder Scarlett Silver Line SL-1545 (ARIMA, UK) and stored at 4 – 7 °C. All sample analyses were done at least in triplicate.

The used chemicals were of the p.a. grade and were purchased from the Sigma Aldrich company.

Methods

Nitrogen and crude protein content determination

The nitrogen and crude protein were analysed using the Kjeldahl's method **(ISO 1871:2009)**. The samples (1 g) and blank runs were mineralised at 420 °C for 105 min. The distillation was performed on Kjeltec™ 2200 (FOSS, Denmark) for 4 minutes. The protein content was calculated using nitrogen-to-protein conversion factor of 6.25.

Fat content determination

The fat content determination was performed by extraction using Soxhlet method **(Soxhlet, 1879)** on the Gerhardt Soxtherm SOX414 (C. Gerhardt GmbH & Co. KG, Germany). Approximately 5 g of dried and homogenized samples (with the accuracy of 0.0001 g) were put into extraction thimbles and extracted by 150 ml of petroleum ether via cold water extraction (program: 70 °C for 120 minutes). The extraction flask was then dried at 103 °C and weighed until a constant sample weight was attained.

Fatty acid profile determination

The esterification of lipids extracted form samples of insects via the Soxhlet extraction was performed according to the **ISO 12966-2:2011** standard using 0.25 M methanolic KOH (test weight of fat for esterification was 0.5 g). Methyl esters of fatty acids were analysed by GC Agilent 7890 (Agilent Technologies, USA) with a flame ionization detector (detector temperature: 250 °C) equipped with a RestekRt®-2560 column (100 m × 0.25 mm ID × 0.2 µm film) from Restek Corporation. Hexane was used as a solvent and the sample volume of 1 μL was injected in split mode (ratio 20:1) into the injector heated to 225 °C. The initial oven temperature was 70 °C (hold 2 min), ramp1 to 225 °C at 5 °C/min (hold 9 min), ramp2 to 240 °C at 5 °C/min (hold 15 min). Helium was used as carrier gas with the flow rate of 1.2 mL/min. The methylated fatty acids were identified using a Restek Food Industry FAME mix (cat#35077). Real chromatogram of Restek Food Industry FAME mix is shown in Figure 1. The proportions of fatty acids were calculated using the area normalisation method.

Statistical analysis

The data were analysed using Excel 2013 (Microsoft Corporation, USA) and the results were expressed by means ±standard deviations.

RESULTS AND DISCUSSION

Crude protein and fat contents of the three investigated edible insect species are shown in Table 1. The obtained values of crude protein in tested insects ranged from 390 to 630 g.kg^{-1} DM. The protein content of *Tenebrio molitor* was found to be higher than in the studies of **Bernard, Allen and Ullrey (1997); Finke (2002); Ramos-Elorduy (2006); Oonincx and Dierenfeld (2012); Yi et al. (2013)** or **van Broekhoven et al. (2015)**. It was also higher than the levels reported by **Bednářová et al. (2013)** who measured the nutrient content of insects bought from a local Czech supplier. The protein content of *Alphitobius diaperinus*is found in this study were consistent with the results reported by **Yi et al. (2013); Bosch et al. (2014)** and **van Broekhoven et al. (2015)**.

Table 1 Lipid and crude protein contents of three edible insect species.

species	crude protein	lipids
	g.kg^{-1} DM ±SD	
Giant mealworm *(Zophobas morio)*	390 ±1	390 ±4
Mealworm *(Tenebrio molitor)*	630 ±4	170 ±1
Lesser mealworm *(Alphitobius diaperinus)*	600 ±5	290 ±3

Table 2 Fatty acid profile of analysed samples.

Fatty acid composition	TM (%)	ZM (%)	AD (%)
SFA			
C8:0	<0.1	1.8	<0.1
C10:0	<0.1	0.4	<0.1
C12:0	0.2	0.1	0.1
C13:0	0.1	<0.1	<0.1
C14:0	3.5	1.7	1.4
C15:0	0.2	0.4	0.3
C16:0	18.4	30.2	26.4
C17:0	0.3	0.7	0.7
C18:0	6.6	8.8	10.9
C19:0	0.1	0.1	0.2
C20:0	0.3	0.2	0.6
C22:0	0.1	0.1	<0.1
Sum of SFA	29.7	44.6	40.6
MUFA			
C14:1, cis - 11	0.1	<0.1	<0.1
C16:1, trans - 11	0.1	<0.1	<0.1
C16:1, cis - 9	1.4	0.7	1.1
C17:1, cis - 10	0.1	0.2	0.2
C18:1, trans - 9	<0.1	<0.1	0.2
C18:1, cis - 9	36.5	31.1	35.9
C20:1, cis - 11	0.1	0.1	0.4
Sum of MUFA	38.4	32.1	37.8
PUFA			
C16:2, trans - 7,10	0.3	1.1	0.3
C 18:2, trans - 9,12	<0.1	<0.1	0.2
C18:2, cis -9,12	30.5	21.2	20.2
C 20:2, cis - 11,14	<0.1	<0.1	0.2
C18:3, cis - 9,12,15	1.1	0.9	0.4
C 20:4, cis - 5,8,11,14	<0.1	<0.1	0.4
Sum of PUFA	31.8	23.2	21.6

Note: TM - larvae of *Tenebrio molitor*, ZM - larvae of *Zophobas morio*, AD - larvae of *Alphitobius diaperinus.*

Figure 5 Giant mealworm *(Zophobas morio)* **(Karwath, 2005)**.

Figure 6 Mealworm *(Tenebrio molitor)* **(Halasz, 2008)**.

Figure 7 Lesser mealworm *(Alphitobius diaperinus)* **(USDA-ARS-GMPRC, 2016)**.

Information about this species reared in the Czech Republic is not available. Compared to traditional protein sources in human nutrition, the content of proteins of both above mentioned species *T. molitor* and *A. diaperinus* are comparable to beef loin (640 g.kg^{-1} DM) or beef flank (640 g.kg^{-1} DM) **(Pipek, 1995; Steinhauser, 1995)**. Crude protein content of the giant mealworm (390 g.kg^{-1} DM) was lower than the contents reported by **Barker, Fitzpatrick and Dierenfeld (1998); Finke (2002); Yi et al. (2013); Bosch et al. (2014)** and also the only known Czech author dealing with this issue **Bednářová et al. (2013)**. On the other hand, the protein content was similar to that determined by **van Broekhoven et al. (2015)**. In comparison with the conventional food, this species could be considered similar to roast pork (410 g.kg^{-1} DM) **(Pipek, 1995; Steinhauser, 1995)**. The differences between obtained results and other studies could probably be caused by using different feeding mixtures or analysing different developmental stages of the sampled larvae.

While the protein contents of the investigated insects varied significantly, the lipid content (Table 1) was found in a range of 170 – 390 g.kg^{-1} DM. The highest lipid content was found in the larvae of the giant mealworm. The analysed lipid content of the giant mealworm was similar to the findings of other papers **(Finke, 2004; Bednářová et al., 2013; van Broekhoven et al., 2015)**. The fat content of *T. molitor* was lower than previously published works suggested. Higher values were reported by **Finke (2004); Bednářová et al. (2013); Yi et al. (2013)** and **van Broekhoven et al. (2015)**. The samples of lesser mealworm contained about fifty grams more lipids than **van Broekhoven et al. (2015)** reported. They discovered the possibility of changes in fat content (up to 10 %) to be caused by feed mixture changes. Therefore, the differences between results of this study and other reported values could be caused by the variety of used feed. In terms of lipid content, all tested species are comparable to a number of traditional foods such as eel meat (300 g.kg^{-1} DM), pork rump (320 g.kg^{-1} DM) or young goose meat (360 g.kg^{-1} DM) **(Pipek, 1995; Steinhauser, 1995)**.

From a nutritional point of view, fatty acid content is very important. Our results in Table 2 show the fatty acid profiles of the fat extracted from the giant mealworm – larvae of *Zophobas morio*, mealworm - larvae of *Tenebrio molitor* and lesser mealworm - larvae of *Alphitobius diaperinus*. Real chromatogram samples of fatty acids composition for all selected insect species are shown in Figure 2, Figure 3 and Figure 4. The recommended ratio of fatty acids for human nutrition is SFA : MUFA : PUFA 1.25 : 1.5 : 1, but the ratio found in *Zophobas morio* is 1.9 : 1.4 : 1. The determined MUFA : PUFA ratio meets the requirements for human consumption (1.4 : 1), but the amount of SFA is significantly higher. Similar ratio is reported by **Bednářová (2013) and Jabir (2012)** – 2.2: 1.9 : 1 and 2.1 : 1.1 : 1. However, **Barroso (2014)** described a lower ratio. Higher SFA content was also determined in the case of lesser mealworms. On the other hand, **Tzompa-Sosa (2014)** presents a lower ratio (1.4 : 1.6 : 1) in case of these species.

In contrast to these species, the amount of SFA in mealworm was significantly lower. Similar results were published by **Zielinska (2015) and Barroso (2014)**. **Tzompa-Sosa (2014)** reported a significant content of MUFA (1.1 : 2.3 : 1), but on the contrary **Bednářová (2013)** measured a greater amount of PUFA (0.7 : 0.8 : 1).

These differences can be caused by a different type of feed and breeding conditions, which were not fully specified by the authors.

Professional and general public pays considerable attention to the ratio of fatty acids n-6 : n-3, which WHO recommends to be 5 : 1 for human nutrition **(Dostálová, Dlouhý and Tláskal, 2012)**. This ratio has a protective effect against non-infectious civilization diseases. The content of n-6 fatty acids in all species, that we analysed, was significantly higher (the ratios of n-6 : n-3 were 26 : 1 for TM, 22 : 1 for ZM, and 53 : 1 for AD). The giant mealworms were the closest to this requirement. On the other hand, lesser mealworms had the highest ratio from the analysed samples. Therefore, the lesser mealworm does not seem to be a perfect primary nutritional source for a long-term human consumption. However, the fatty acids proportions could be affected by changing the insects' feed composition. Balanced diet of people eating insects is also important.

A higher content of unsaturated (n-9) oleic acid was measured in all samples. The amount of this acid is comparable to the traditional sources such as beef tallow (26 – 50 %), and sheep tallow (30 – 42 %), but **DeFoliart (1992)** reported that composition of fatty acids is similar to poultry and fish. The second most represented fatty acid in giant mealworm and lesser mealworm was palmitic acid (31.1 % and 26.4 %). A similar content of this acid is to be found in rabbit lard (32 %) **(Velíšek, 2002)**. Linoleic acid was the third most abundant fatty acid in these samples (21.2 % and 20.2 %). However, in case of mealworm, the second most represented acid was the essential linoleic acid (30.5 %) and the third was palmitic acid (18.4 %). The highest content of essential α-linolenic acid was also measured in mealworm. Therefore, mealworm could be the most suitable insect of the analysed species for human consumption.

The descending order of the first four minor fatty acids for giant mealworm is the same as reported by **Bednářová et al. (2013)**. However, the ratios found differed slightly. Also, some fatty acids not detected by **Bednářová et al. (2013)** were determined. An example of such is the α-linolenic acid, whose content was measured to be twice the amount of the arachidic acid. Unfortunately, **Bednářová (2013)** did not mention the composition of the feed mixture. Our results are also in line with the data reported by **van Broekhoven et al. (2015)**. The order of the first four acids is identical, while the mutual ratio varied in dependence on feed (i.e. when insects were fed by feed with high starch and low protein content, the ratio between linoleic and oleic acid was 0.22 : 1; when they were fed by high-protein and low-starch feed this ratio changed to 0.94 : 1).

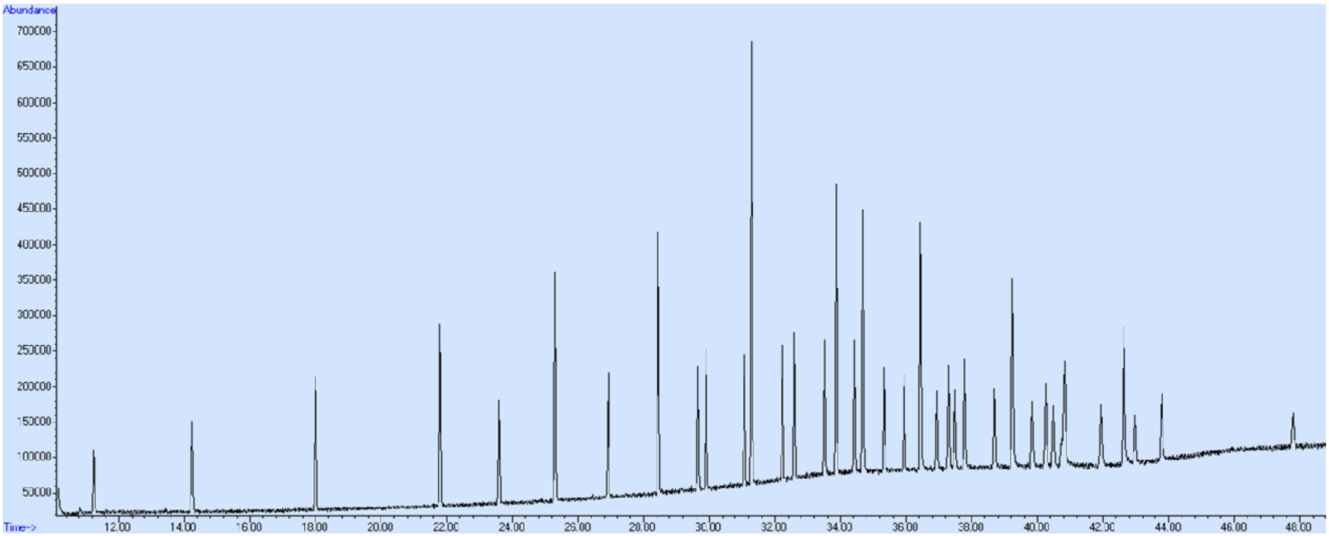

Figure 1 Chromatogram - fatty acids composition of Restek Food Industry FAME mix (cat#35077).

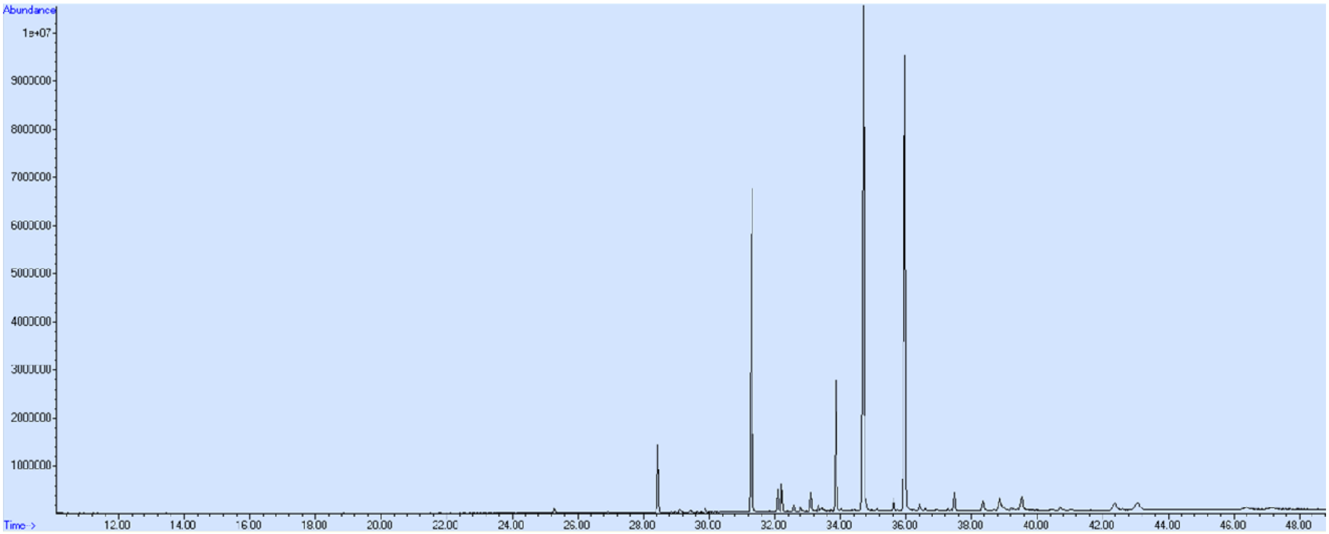

Figure 2 Chromatogram – fatty acids composition of mealworm – larvae (*Tenebrio molitor*) reared in the Czech Republic.

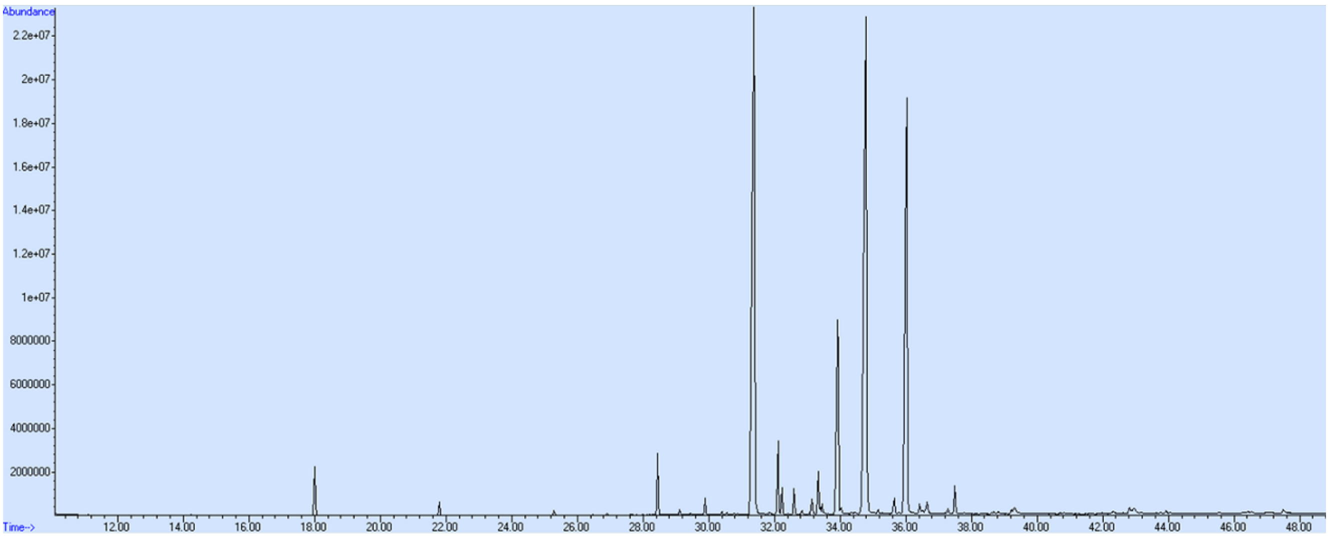

Figure 3 Chromatogram – fatty acids composition of giant mealworm – larvae (*Zophobas morio*) reared in the Czech Republic.

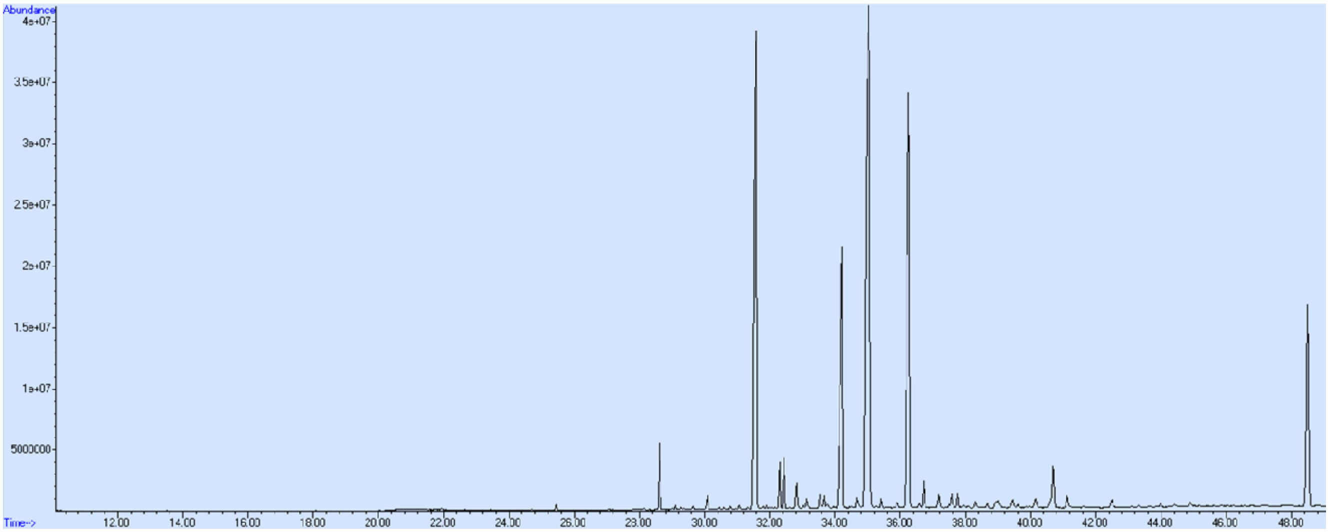

Figure 4 Chromatogram – fatty acids composition of lesser mealworm – larvae (*Alphitobius diaperinus*) reared in the Czech Republic.

CONCLUSION

This work was focused on the nutritional composition determination of three edible insect species reared in the Czech Republic. Based on the obtained results, all investigated species (*Zophobas morio*, *Tenebrio molitor* and *Alphitobius diaperinus*) could be considered as a reasonable source of lipids and two of them (mealworm and lesser meal worm) are also an excellent source of proteins. The results of fatty acids profile of the giant mealworm and lesser mealworm showed that they are not very suitable as the main food ingredient due to a high SFA content and an inappropriate n-6 and n-3 ratio. Out of all measured samples, mealworm has the highest content of linoleic and α-linolenic acid, which are among essential components of human nutrition.

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