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IS EDIBLE INSECT AS A NOVEL FOOD DIGESTIBLE?

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ABSTRACT

This work deals with the digestibility of a selected species of edible insect - mealworm (larvae) as novel food in dependency on its culinary treatment. The aim of this work was to find suitable thermic culinary treatment of mealworm larvae considering its optimum digestibility by human. The digestibility of materials from whole insect and extracted nitrogenous substances was determined using three different culinary treatments - without culinary treatment (freshly killed), dried insect and roasted insect. The digestibility was determined by gravimetric in vitro method using pepsin and pancreatin enzymes and their combination. The total nitrogen content of the insect samples was determined by the Kjeldahl method. The digestibility of the whole homogenized larvae using the combination of pepsin and pancreatin enzymes, thus simulating human digestion in-vitro, ranged from 81% for roasted specimens to 91.5% for culinary unprocessed insect. Similarly, the digestibility of nitrogenous substances of homogenized insect samples using this combination of enzymes ranged from 24.2% for roasted specimens to 80.2% for culinary unprocessed samples. The work showed the dependence of the digestibility of the mealworm larvae on the culinary treatment - the increasing heat load of the sample reduced the digestibility. Furthermore, it proved the effect of the digestive enzyme on the digestibility of the insect sample.

Keywords: digestibility; mealworm; culinary treatments; enzymes; nitrogenous substances

INTRODUCTION

Digestion is a physiological process in which nutrients contained in food are decomposed into a resorbable form. Nitrogenous substances, fats and carbohydrates have to be split up so that they can pass through the intestinal wall into the blood. The blood will transport them further to the necessary places in the organism where they are utilized **(Mišurcová et al., 2010)**. Digestibility is most commonly determined as protein digestibility. To a large extent, this digestibility is influenced by the culinary treatment. Culinary treatment, especially cooking and frying, improves sensory quality of food, and induces formation of flavours, attractive colours and textures. Cooking also improves hygienic quality by inactivating some pathogenic microorganisms, improves digestibility and increases the bioavailability of certain nutrients in the gastrointestinal tract **(Bognár, 1998)**.

At present, many studies **(Megido et al., 2018; Grabowsky and Klein, 2017; Klunder et al., 2012; Vandeweyer et al., 2017)** deal with the hygiene and food safety conditions applicable in the European food industry for edible insect, but only a few studies deal with the influence of culinary treatment on the edible insect nutritional value. This creates an information gap for everyday consumers, chefs, cookbooks authors, etc., who have minimal access to information about a safe and healthy way to cook edible insect **(Megido et al., 2018)**. Due to the increasing demand for commodities of animal origin, focusing on protein sources and their digestibility, consumer pressure is also increasing to fill this information gap **(Mlček et al., 2014; Tan, Berg and Stieger, 2016; Adámková, 2017)**. In addition, the availability of this information may reduce fears in the part of the European public about the consumption of edible insect **(Yen, 2009)**.

During the heat treatment of food, proteins are denatured, amino acids modified or destroyed and Maillard reaction occurs. In the heat treatment, proteins may also interact with other proteins or with oxidizing agents, sugars, polyphenols, tannins or solvents **(Finot, 1983)**. Denaturation at higher temperatures results to better enzymatically digestible proteins due to cleavage of developed polypeptide chains or inactivation of antinutritional compounds **(Finot, 1983; Opstevedt et al., 2003)**. On the other hand, the digestibility of proteins may be reduced by reacting with each other and by reacting with amino acids which cannot subsequently be hydrolysed by digestive enzymes **(Opstevedt et al., 2003)**.

The question of the use of edible insect as part of feed in livestock and pets (dogs, cats, etc.) has been dealt with by several studies **(Bosch et al., 2014; McCusker et al.,**

2014; De Marco et al., 2015; Panini et al., 2017). In spite of these data, the knowledge about digestibility of edible insect in humans is minimal. The reason is physiological differences and differences in the composition of digestive juices, therefore the digestibility of this commodity may be different in man and animal **(Bussink et al., 2007)**. Due to the inclusion of edible insect in the "novel food" category in European countries, the solution to this issue becomes important when a complex view of edible insect is needed, concerning not only nutritional or sensory properties, but also the digestibility.

For this reason, this study focused on digestibility of edible insect, which assumes that digestibility is different for different culinary treatments of insect. The aim was to find a suitable heat culinary treatment of the mealworm in terms of its optimum digestibility by man. Because of the inclusion of edible insect in the novel food category, comparison is also required with other commodities of animal origin. For this reason, this study focused on digestibility of edible insect, which assumes that digestibility is different for different culinary treatments of insect

Scientific hypothesis

Scientific hypothesis is: the digestibility of edible insect materials is dependent on culinary treatments. The aim was to find a suitable heat culinary treatment of the mealworm in terms of its optimum digestibility by man. Because of the inclusion of edible insect in the novel food category, comparison is also required with other commodities of animal origin.

MATERIAL AND METHODOLOGY

Material

For the analysis, samples of mealworm larvae (*Tenebrio molitor*) were used for analysis. Samples were purchased at a pet store. Prior to analysis, insect samples were treated as follows: mealworm larvae in the last and penultimate stages were taken from the breed and left to starve for 24 hours. Subsequently, the insect was killed with boiling water
(100 °C) and dried with a warm air stream at a temperature of 75 °C ±5 °C for 30 s. Samples of killed and wiped larvae were divided into three experimental groups with the following treatment procedures:

1. no treatment – freshly killed insect with no further culinary treatment
2. dried insect – killing, subsequent drying for 2 minutes at 120 °C and then drying for 5 – 7 minutes at 70 – 80 °C
3. roasted insect – killing, subsequent roasting for 4 minutes at 160 °C.

After treatment, all samples were homogenized and stored in cooling box at 4 – 7 °C until analysis.

Dry matter digestibility determination

Determination of digestibility was performed by gravimetric in vitro method using a Daisy incubator (ANKOM Technology, USA). For digestion, pepsin EC 3.4.23.1 from porcine gastric mucosa (activity: 0.7 FIP-U.g^{-1}) and pancreatin from pancreas (protease activity: 350 FIP-U.g^{-1}, lipase activity: 6000 FIP-U.g^{-1}, amylase activity: 7500 FIP-U.g^{-1}) were used. Both enzymes were supplied by Merck (Darmstadt, Germany).

Enzymatic hydrolysis involved hydrolysis by pepsin (0.5 g enzyme per g sample), pancreatin (0.5 g enzyme per 1 g of sample) and combined hydrolysis with pepsin and subsequently with pancreatin. In case of hydrolysis by pepsin, digestibility was measured after 30 minutes. For pancreatin hydrolysis, digestibility was determined after 6 hours. In the case of combined hydrolysis, the pepsin enzyme was left to function for 30 minutes, followed by the pancreatin enzyme treatment for 6 hours. Samples were evaluated 3 times. The determination was carried out according to the modified methodology **(Mišurcová et al., 2010; Mišurcová, 2008)**.

For determination of digestibility, 0.5 g of sample was weighed into F57 filter bags with a porosity of 25 μm (ANKOM Technology, USA). The bags were sealed, placed in incubation flasks containing 1.7 liters of the appropriate solution (in the case of pepsin 0.1 M HCl, in the case of pancreatin pH 7.45 phosphate buffer), conditioned to 40 °C and added to adequate amount of the corresponding enzyme to meet the above requirement of 0.5 g of enzyme per 1 g of sample. Together with the samples, a sealed control bag without a sample was placed in the incubation bottle. This was followed by hydrolysis for the time intervals mentioned above. After the hydrolysis was complete, the bags were washed with distilled water, dried for 24 hours at 103 °C and weighed. In the case of combined hydrolysis, the samples were first hydrolysed with pepsin, and hydrolysis with pancreatin was initiated immediately after completion of the pepsin hydrolysis and washing of the bags in distilled water **(Mišurcová et al., 2010; Mišurcová, 2008)**.

Determination of nitrogenous substances digestibility

To determine the digestibility of nitrogenous substances, the nitrogen content of the non-hydrolysed samples and the nitrogen content of the samples enzymatically hydrolysed with pepsin, pancreatin and combined – pepsin and then pancreatin – had to be evaluated. Enzymatic hydrolysis was carried out as described above. The total nitrogen content of both hydrolysed and non-hydrolysed insect samples was determined by the Kjeldahl method using an automatic distillation unit Pro Nitro A (JP Selecta S.A., Spain). The results were expressed as a percentage in the form of the coefficient of digestibility of the nitrogenous compounds.

The coefficient of digestibility of nitrogenous compounds (KS) can be calculated according to the equation below (1). To calculate the digestibility coefficient, the nitrogen content of the non-hydrolysed samples (NLN) from equation (2) and the nitrogen content of the hydrolysed samples (NLH) from equation (3) **(Mišurcová, 2008)** must be determined. Samples were measured 2 times.

$$K_S = \frac{NL_N - NL_H}{NL_N} \cdot 100 \quad (1)$$

$$NL_N = \frac{N_N}{m_{NL}} \cdot f \cdot 100 \quad (2)$$

$$NL_H = \frac{N_H}{m_{NL}} \cdot f \cdot 100 \quad (3)$$

where:

K_S digestibility coefficient (%),
NL_N content of nitrogenous substances in non-hydrolysed samples (%),
NL_H content of nitrogenous substances in hydrolysed samples (%),
N_N content of nitrogenous substances determined by Pro Nitro in non-hydrolysed samples (mg),
N_H content of nitrogenous substances determined by Pro Nitro in hydrolysed samples (mg),
m_{NL} sample weight (mg),
f conversion factor (f = 6.25).

Statistic analysis

Data was evaluated using Excel 2013 (Microsoft Corporation, USA) and STATISTICA Cz version 12 (StatSoft, USA). The results were expressed by average ± standard deviation. Kruskal-Wallis test (α = 0.05) was used to compare of samples.

RESULTS

The samples were hydrolysed with pepsin, pancreatin, and their combination (marked as "PePa"). The digestibility of the dry matter for each sample is shown in Table 1.

The highest digestibility was found in untreated samples. With processing, the digestibility decreased. The lowest was found for roasting, which can produce enzymatically unprocessable complexes. For the pepsin enzyme and dried and roasted samples, this value decreased by more than 35%. The pancreatic enzyme and combination of enzymes did not make such difference - for pancreatin, it was less than 15% and less than 11% for enzyme combination. The dried sample hydrolysed by the combination of pepsin and pancreatin enzymes has an average value just slightly below the level of the sample hydrolysed only by the pancreatin, and it seems that the above trend cannot be applied.

In the case of monitoring the dependence on the type of hydrolysis after the same heat treatment, it was found that the lowest digestibility values were determined for the pepsin enzyme, Figure 1. The reason is the chosen hydrolysis time (30 min). On the other hand, despite this hydrolysis time, the digestibility of unprocessed insect was more than 85%. In hydrolysis by the pancreatin enzyme, where the hydrolysis time was longer, the digestibility was determined to be up to 30% higher. The highest digestibility values were reached by the combination of pepsin and pancreatin. In this case, digestibility was over 80% for all culinary treatments (no processing, drying and roasting). This combined hydrolysis is most similar to human digestion from the hydrolysis types used in this work.

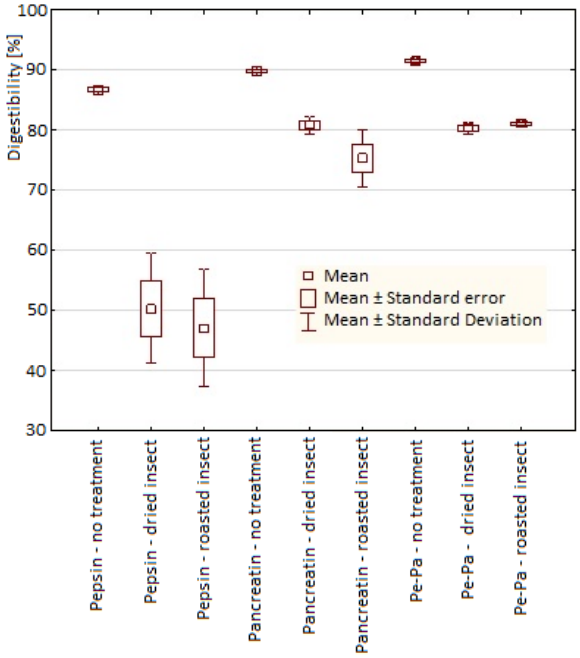

Figure 1 Digestibility of samples enzymatically hydrolyzed with pepsin, pancreatin and combined – pepsin and then pancreatin (marked as "PePa").

Due to the non-compliance with the homogeneity condition for some sample sets, the Kruskal-Wallis test and the multiple comparison of the *p-values* were selected for the comparison of the groups. The results of comparison of the groups are shown in Table 2. In this table a statistically significant difference between roasted and untreated samples by pepsin hydrolysis can be seen. A statistically significant difference (p <0.01) between unprocessed and roasted samples can also be found in pancreatin hydrolysis. In hydrolysis by the combination of these enzymes, a statistically significant difference was found between the dried and untreated samples. No other statistically significant difference was found in this study, although some differences can already be traced from the chart.

For each sample gained by hydrolysis the content of crude protein was analysed, Table 3. This value was used to calculate the digestibility of the nitrogenous substances. From the measured values of nitrogenous substances for individual samples, their digestibility was determined, Table 4. In this table, a significant decrease in the digestibility of nitrogenous substances in hydrolysed

Table 1 The digestibility of samples [g.100g^{-1}].

	no processing		dried insect		roasted insect	
	M	SD	M	SD	M	SD
Pepsin	86.7	0.8	50.4	9.2	47.2	9.8
Pancreatin	89.8	0.7	80.8	1.4	75.3	4.7
Pe-Pa	91.5	0.6	80.3	0.9	81.0	0.5

Note: PePa – combined hydrolysis using pepsin and pancreatin.

Table 2 Multiple comparison of the *p-values* for different culinary treatments and hydrolyses with pepsin, pancreatin and their combination.

	Pepsin		
Dependent value	Multiple comparison of the *p-values* (both sides) Kruskal-Wallis test: H =7.423077; *p* = 0.0244		
	No treatment	Drying	Roasting
No treatment		0.072337	0.042684
Drying	0.072337		1.000000
Roasting	0.042684	1.000000	
	Pancreatin		
Dependent value	Multiple comparison of the *p-values* (both sides) Kruskal-Wallis test: H = 9.846154; *p* = 0.0073		
	No treatment	Drying	Roasting
No treatment		0.349993	0.005106
Drying	0.349993		0.349993
Roasting	0.005106	0.349993	
	Pepsin + Pancreatin		
Dependent value	Multiple comparison of the *p-values* (both sides) Kruskal-Wallis test: H = 8.000000; *p* = 0.0183		
	No treatment	Drying	Roasting
No treatment		0.018119	0.149581
Drying	0.018119		1.000000
Roasting	0.149581	1.000000	

samples with culinary treatment can be seen. It is believed that the decline in digestibility is due to the formation of enzymatically unprocessable complexes due to the increasing heat effect of heat culinary treatment.

DISCUSION

Several parameters can affect digestibility, e.g. chitin content, phytate content, interaction of individual nutrients, oxidative changes, etc. The results are simulated in vitro, so they can be different from real digestive processes **(Svačina, 2010)**. **Poelaert et al. (2016)** determined the digestibility of unprocessed mealworm dry matter by in-vitro method (IVDMD) 76.2%. This result is lower than in this work. Similarly, this was also the case with thermal effects on commodities, where **Poelaert et al. (2016)** declared an 18% lower digestibility than that measured in this work. However, the trend is similar in both researches. In accordance with this work, **Poelaert et al. (2016)** noticed reduced protein digestibility when using a heat processing of up to 13% when samples were autoclaved.

When comparing with mealworm, **Poelaert et al. (2016)** declared up to 23% lower digestibility of the house cricket

Table 3 Nitrogenous substances content in samples [g.100g^{-1}].

	no processing		**dried insect**		**roasted insect**	
	M	**SD**	**M**	**SD**	**M**	**SD**
No hydrolysis	204.2	1.7	739.4	24.8	488.0	2.1
Pepsin	58.8	4.4	668.8	0.8	184.2	2.5
Pancreatin	54.0	3.2	618.9	8.6	171.9	1.4
Pe-Pa	40.5	1.2	560.3	11.0	149.2	0.9

Note: PePa - combined hydrolysis using pepsin and pancreatin.

dry matter depending on the heat treatment. However, protein digestibility (IVCPD) is comparable in both species. **Poelaert et al. (2016)** also reports a comparison with commodities of plant origin (beans, lentils, peas, soybean), where the digestibility is mostly lower in raw state and the significantly increases with raising temperature - the lentils had an increase in digestibility by up to 28%. Generally, however, the digestibility of dry matter in these commodities of plant origin is up to tens of % lower than determined by **Poelaert et al. (2016)** in their work for a mealworm or than the values in this study.

In terms of nutritional values, however, the more important is the digestibility of crude proteins determined in vitro (IVCPD). Besides **Poelaert et al. (2016)** also **Marono et al. (2015)**, **Caparros Megido (2017)**, and **Panini et al., (2017)** dealt with it. **Panini et al., (2017)** for his research on "alternative protein source for Pacific white shrimp" reported a 45.9% dry matter digestibility and 76.1% protein digestibility for "mealworm meal". **Marono et al. (2015)** declared the protein digestibility of "insect meals" from different suppliers ranging from 65.5% to 66.7%. These values are comparable to the values (59.5% – 72.5%) reported by **Poelaert et al. (2016)** and values measured in this work but, are lower than the values (85.0% – 91.5%) reported by **Megido et al. (2018).** Although the difference in digestibility between **Poelaert et al. (2016)** and **Megido et al. (2018)** was 13% for a crude insect sample, **Poelaert et al. (2016)** declared it as the highest, and **Megido et al. (2018)** as the lowest. From the results reported by **Megido et al. (2018)**, therefore, the trend is the increasing protein digestibility with raising the temperature. On the contrary, **Poelaert et al. (2016)** show the opposite trend - heat treatment reduces protein digestibility. This trend can also be seen for the results in this work. However, the specific values are not completely comparable, due to different experimental methodology (e.g. time and temperature of hydrolysis, selected enzyme

Table 4 Digestibility of nitrogenous substances after culinary treatment and hydrolysis with the selected enzyme.

Sample	Nonhydrolyzed sample [g.100g^{-1}]	Hydrolyzed sample [g.100g^{-1}]	Digestibility [%]
No treatment			
Pepsin	204.2	58.8	71.2
Pancreatin	204.2	54.0	73.5
Pepsin and pancreatin	204.2	40.5	80.2
Drying			
Pepsin	488.0	184.2	62.3
Pancreatin	488.0	171.9	64.8
Pepsin and pancreatin	488.0	149.2	69.4
Roasting			
Pepsin	739.4	668.8	9.5
Pancreatin	739.4	618.9	16.3
Pepsin and pancreatin	739.4	560.3	24.2

type, correction). For this reason, it is possible to compare only culinary treatments between themselves and the influence of a particular enzyme.

When comparing digestibility with samples of animal origin, **Megido et al. (2018)** pointed out the match of their results with other commodities - beef (89%), pork (90%), turkey meat (78%) and salmon (85%) **(Bodwell, Satterlee and Hackler, 1980)**. They declared the differences from other studies were due to the different "raw materials" and the use of various "different batches of mealworms" with different fat or antinutritional factors content. At higher temperatures, digestibility is reduced as a result of the formation of difficult-to-digest protein complexes with oxidized fats. In addition, digestibility can be reduced by, for example, reacting with mineral substances and reacting minerals with one another. Reagents, such as phosphorus and calcium, form an insoluble complex (phytates) that reduces the digestibility of proteins and makes them inaccessible **(El Hassan et al., 2008).**

Similar to other commodities, the heat can not only positively affect the properties, but can also lead to a reduction in nutritional value, e.g. by oxidation of amino acids or by changing or losing essential amino acids, or even creating substances that are undesirable from the point of view of health (toxic, carcinogenic or mutagenic effects substances). Highly dangerous substances can arise from proteins of animal origin (i.e. insect), and therefore all excessively browned to blackened portions of the food should be removed. Insect, in our case, mealworm is a specific biological material. Despite being regarded a farm animal after being included into novel foods by EFSA, it has a different anatomy and physiology of the body than ordinary livestock (mammals). Therefore, it should be borne in mind that, from the nutritional point of view, this commodity contains, in addition to fat and crude protein, a considerable amount of chitin **(Adámková et al., 2017)**. However, the European consumer does not have enough chitinase to digest it.

CONCLUSION

The digestibility of edible insect, on which this work was focused, is dependent on subsequent culinary treatments. In terms of the digestibility of the dry matter, the highly in-vitro digestible sample of the mealworm is thermally untreated and the most difficult for digesting is sample after roasting. However, for the safety reasons, it is not possible to recommend the consumption of unprocessed mealworm meal by humans. However, insect can be used both as dried and uncooked (freshly killed) as feed for farm animals. Even in the case of nitrogen digestibility analysis, the highest digestibility value was detected for thermally unprocessed insect. From a safety point of view, the heat treatment by drying is more suitable, which reduces the digestibility of nitrogenous substances, but not so much as in the case of roasting. The practical use of this work lies in the contribution of knowledge that could enable the fortification of food by the addition of commodity from edible insect ideally roasted. However, due to the possible formation of dangerous roasting complexes (Maillard reaction), further analyses are needed in this area.

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