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FATTY AND AMINO ACID PROFILE OF MEALWORM LARVAE (*TENEBRIO MOLITOR* L.)

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Abstract: The yellow mealworm (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) is an edible insect, distributed worldwide and a convenient candidate for industrial-scale production. Mealworms could be commercially used for the substitution of conventional protein sources. In our previous study, it was found that *T. molitor* larvae predominantly contained crude protein (55.83%) and crude fat (25.19%), as well as low content of nitrogen-free extract (based on dry weight). Mealworm specimens were maintained in an incubator under controlled conditions in plastic containers. Insects were sieved and put into the container with boiling water and cooked for 180 seconds. Moisture content was determined as weight loss after drying of larvae. Amino acids were determined on an Agilent Technologies 1260 series HPLC system. Fatty acid composition was determined on a Thermo Scientific TRACE 1300 gas chromatograph equipped with a flame ionization detector using TR-FAME column. The results showed that the content of unsaturated fatty acid is very high, i.e. oleic acid (C18:1) formed the major lipid component in 40.83%, which was followed by linoleic acid (C18:2, omega-6 fatty acid) with 29.80% and linolenic acid (C18:3) with 1.08%. The essential amino acids are highly represented in the samples (in % dry matter). This primarily refers to isoleucine (4.12), tyrosine (3.86), phenylalanine (3.06), leucine (2.96), lysine (2.67) and methionine (1.76). The differences in essential fatty and amino acid content between our results and discussed literature data, could be the consequence of different substrates used for rearing of insects. After everything stated above, the biological value of *T. molitor* larvae proves that it could be suitable as animal feed.

Key words: edible insects, *Tenebrionidae*, chemical composition

Introduction

The world population is anticipated to reach 9 billion by 2050 and the challenge to feed the increasing population is huge, given the limited agricultural resources of land and water in the climate-change era (*FAO, 2009*). Thus, safeguarding sustainability has become the most pertinent challenge of today. In this context, insects could be viewed as an alternative source of animal feed and human food in terms of micro-livestock. Edible insects have played an important role in the long history of human nutrition in Africa, Asia and Latin America *(Aletor, 1995)*. More than 1000 species of insects that are edible in a certain stage of their life cycle have been reported worldwide as traditional foods by humans and has been an important part of the nutritional intake and economic value of many societies *(Illgner and Nel, 2000)*. Not every edible insect species contains every nutrient. There is scope for selective breeding and possibilities exist to fortify species with nutrient components they are lacking or to prepare a mixture of different insects as an ideal nutritional source used in insect flours or as food supplements. Inclusion of insects in the animal meat product industry should ease the excessive pressure on conventional livestock production and in turn on the environment *(Gnosh et al., 2017)*.

The yellow mealworm (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) is an edible insect, distributed worldwide and a convenient candidate for industrial-scale production (*Van Huis, 2013; EFSA, 2015)*. Mealworm larvae are commercially used as animal feed and in some countries, such as Asia and Africa, for human nutrition because of their high fat, protein and mineral content *(Finke, 2002; Rumpold and Schluter, 2013).* Even though *T. molitor* are insects that infest stored products, many people do not consider them as pests, but culture their larvae in large quantities for sale as food for insectivorous animals (such as birds and aquarium fishes) raised in captivity *(Ng et al., 2001). T. molitor* are the most widely reared insects as human food in Europe *(Caparros Megido et al., 2014). Ravzanaadii et al. (2012)* suggested that mealworms could be commercially used for the substitution of conventional protein sources (as they contain approximately 46% proteins based on dry weight). The authors also indicated that these insects contain approximately 33% lipids (dry weight basis). According to the results of *Siemianowska et al. (2013)* the fresh mealworm larvae contained more total protein, total fat and ash in comparison to traditional meats i.e. chicken, pork, beef, fish and eggs. In our previous study, it was found that *T. molitor* larvae predominantly contained crude protein (55.83%) and crude fat (25.19%), as well as low content of nitrogen-free extract in dry matter *(Jajić et al., 2019).*

High content of fatty acids in diets affects their antioxidant activity, which is highly desirable in human diets *(Wojciak and Dolatowski, 2012). Yang et al. (2006)* found that edible insects contained good quality fatty acid especially long

chain omega-3 fatty acids such as alpha-linolenic acid, eicosapentaenoic acid and that different kinds of insects had different fatty acid profiles. Mealworms contain high amounts of unsaturated fatty acids, mainly linoleic acid and oleic acid, and palmitic acid as saturated fatty acid *(Lenaerts et al., 2018). Paul et al. (2017)* concluded that oleic and linoleic acids formed the major fatty component of *T. molitor* larvae and *Acheta domesticus* lipids, respectively. These results were in good agreement with the findings of *Ravzanaadii et al. (2012) and Tzompa-Sosa et al. (2014)*. Even though both these insect species were fed with a similar diet (containing wheat flour, wheat bran and brewer's yeast), they exhibited a different fatty acid composition. This indicates that fatty acid profile also varies with individual species. Almost all insects are able to biosynthesize palmitic, stearic and oleic acids *(Stanley-Samuelson et al., 1988; Grapes et al., 1989)*. *T. molitor* larvae lipids exhibit a very high n-6/n-3 ratio *(Bendová et al., 1991).* In the study of *Nielsen (2016),* it was indicated that omega-3 fatty acids in *T. molitor* larvae lipids could be enhanced by omega-3 fatty acid supplementation in the diet. In the study of *Janssen et. al. (2017)*, the fatty acid analysis showed an extraordinary composition of long chain fatty acids (C18-C24), oleic acid (C18:1) being the main component followed by linolenic acid (C18:2) and palmitic acid (C16). Significant amounts of linolenic (C18:3) were also found. *Nielsen (2016)* indicated that omega-3 fatty acids in *T. molitor* larvae lipids could be enhanced by omega-3 fatty acid supplementation in the diet. *Ravzanaadii et al. (2012*) found remarkable content of long chain fatty acids (C18-C22) with oleic acid (C18:1), linoleic acid (C18:2) and palmitic acid (C16) being the highest components. In addition, comparatively high amounts of omega-3 fatty acids were found in larvae. These essential fatty acids are mostly available in sea species, where mealworms are demonstrating that it can be used for many other purposes such as feeding of domestic animals, food supplement and recycling supplement, etc. (*Nettleton, 1995; Ravzanaadii et al., 2012). Ravzanaadii et al. (2012)* found considerable amounts of unsaturated fatty acids (77.74%). In terms of degree of unsaturation of fatty acids, insects have composition similar to poultry and fish. In some groups, such as essential fatty acids, linolenic acids and linoleic were even higher than fish and poultry *(Defoliart, 1991). T. molitor* larvae were characterized by favorable proportion of n-6/n-3 fatty acids in comparison to pork meat. A very good n-6/n-3 acids ratio (6.76) in mealworm larvae may be taken as another determinant of their high quality and nutritive value.

All insect species considered as animal feed have high levels of protein with amino acid profiles suitable to be used as feedstuffs *(Makkar et al., 2014; Henry et al., 2015; Veldkamp and Bosch, 2015; Van Huis and Tomberlin, 2017)*. Furthermore, insects can be used as a natural nutrient source for poultry *(Jozefiak et al., 2016)*. The amino acid composition of proteins determines their quality as animal feed. The optimal amino acid composition may vary among different species due to different feed requirements and some amino acids may need to be

supplemented *(Müller et al., 2017)*. It has already been proven that edible insects may provide sizable amounts of protein, including all indispensable amino acids *(Ramos-Elorduy et al., 1997; Verkerk et al., 2007; Chen et al., 2008)*. Overall, insects contain higher amounts of lysine and threonine which are deficient in most commonly used wheat, rice, cassava and maize, but lower amounts of methionine and cysteine *(Defoliart, 1992)*. *Finke (2004)* stated that adult specimens of *T. molitor* contain 653 g protein per kg dry matter (DM), while larvae of the same species contain 49.1 g protein per kg DM. In addition, the digestibility of insect protein is comparable to that of meat protein *(Verkerk et al., 2007; Longvah et al., 2011)*. Insects are therefore presented by the Food and Agriculture Organization of the United Nations (FAO) as a valuable alternative to meat *(FAO, 2013)*. Conventional meat contains high levels of purines *(Choi, 2010)*, which are very important to the human body, as two of the nucleic acids, adenine and guanine, are purines *(Bednarova et al., 2013)*. In the research of *Aguilar-Miranda (2002)* the amino acid content of *Tenebrio* larvae powder showed the requirements of essential amino acids as reported by *FAO/WHO/UNU (1986)*. Larval powder had high phenylalanine + tyrosine (7.7 g/100 g of protein) and tryptophan contents (1.8 g/100 g of protein).

The aim of the present study was to determine the fatty and amino acid composition of the powdered *T. molitor* larvae.

Material and Methods

Rearing of insects

The insects were obtained from the Department of Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Serbia. Mealworm specimens were maintained in an incubator under controlled conditions (temperature: 27±1 °C, photoperiod: 0 h light - 24 h dark, relative humidity: 55%) in 12 L plastic containers (20 cm x 40 cm x 15 cm). The insects were grown on a food mixture which contained 400 g of wheat bran, 250 g of dried barley germs, 200 g of dried oat germs, 50 g of barley flakes, 50 g oat flakes and 50 g of powdered beer yeast. Pieces of apple were spread over the food mixture to provide additional moisture to the insects.

Preparing insects for drying and cooking

Insects were sieved (2.5 mm pore diameter) and the remaining insect parts were removed with weak wind flow produced by hair dryer. Sieved larvae were moved to the sieve with smaller holes and with weaker windflaw remains of insect bodies were removed. Afterwards cleaned larvae were transferred into the 2 L

plastic container, and gently washed under the water jet. After that insects were put into the container with boiling water and cooked for 180 seconds. Thereafter, the entire content of the cooking pot was filtered through sieve in order to remove water, and then, the larvae were spread onto the filter paper in a thin layer in order to evaporate excessive water during 24 h. Dried insects were collected and put on a new filter paper and left to dry for another 24 h.

Chemical analysis

Dry matter content (DM) was determined after drying (AOAC Official Method 934.01). Crude protein (CP) was analyzed according to standard Kjeldahl method (AOAC Official Method 2001.11), while crude fat content (EE) was determined as petroleum ether extract (AOAC Official Method 991.36).

Amino acids were determined on an Agilent Technologies 1260 series HPLC system (Agilent Technologies, USA) by applying previously established analytical conditions *(Jajić et al., 2013)*. Fatty acid composition was determined on a Thermo Scientific TRACE 1300 gas chromatograph equipped with a flame ionization detector (Thermo Scientific, USA) using TR-FAME (length 30 m, inner diameter 0.32 mm, film thickness 0.25 μm) column (Thermo Scientific, USA). The injector and detector temperatures were 200 °C. Helium was used as carrier gas with a flow rate of 1.3 mL/min. Sample and standard were diluted in n-heptane (analytical purity). 1 μl of sample was injected into the injector. The fatty acid composition was calculated based on the area of the peaks. Prior to GC analysis, fat was extracted from samples using Soxhlet extractor. About 20 mg of fat was weighted in 5 cm^3 v-vial (Sigma-Aldrich, Switzerland) and 0.5 ml of 0.5 M NaOH was added. Vial was then heated to 70 °C for 10 minutes and cooled to room temperature. Then, 0.5 ml of boron trifluoride (Sigma-Aldrich, Switzerland) was added and again heated to 70 °C for 10 minutes and cooled to room temperature. Finally, 1 ml of saturated NaCl solution and 1 ml n-heptane was added and gently mixed. Upper (heptane) layer was transferred into 1 ml tube containing anhydrous sodium-sulfate. After holding for 30 minutes, heptane layer was transferred into GC vial and then analyzed.

Results and Discussion

We were able to identify nine fatty acids present in different proportions. The results in Table 1 were presented in different units for easier comparison with results from previous research.

As can be seen, the content of unsaturated fatty acid is very high, i.e. oleic acid (C18:1) formed the major lipid component in 40.83%, which was followed by linoleic acid (C18:2, omega-6 fatty acid) with 29.8% and linolenic acid (C18:3)

with 1.08%. On the contrary, the amount of saturated fatty acids is generally lower than above mentioned and belonged predominantly to palmitic acid (C16:0) at 16.2% and stearic acid (C18:0) with 2.21%. Other fatty acids from both groups were in individual quantities of less than 1%.

Table 1. Fatty acid profile

Fatty acids	**g/100 g fat or % fat**	**% in sample**	**% DM or g/100 g DM**	**g/100 g protein**	**mg/g protein**
Palmitic acid C16:0	16.20±0.47	4.08±0.12	4.16±0.12	7.44±0.22	74.45±2.16
Stearic acid C18:0	2.21±0.03	0.56±0.01	0.57±0.01	1.02±0.01	10.16±0.14
Oleic acid C18:1	40.83±0.57	10.29±0.14	10.48±0.15	18.76±0.26	187.64±2.60
Linoleic acid C18:2 (omega-6)	29.80±0.46	7.51±0.11	7.65±0.12	13.69±0.21	136.95±2.09
Linolenic acid C18:3	1.08±0.02	0.27±0.01	0.28±0.01	0.50±0.01	4.96±0.11
Eicosanoic acid C20:0	0.09±0.01	0.02±0.00	0.02±0.00	0.04±0.00	0.41±0.03
Docosanoic acid C22:0	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.09±0.00
Eicosapentaenoic acid (EPA); C20:5	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.09±0.00
Docosahexaenoic acid (DHA), C22:6	0.07±0.01	0.02±0.00	0.02±0.00	0.03±0.00	0.32±0.04
Other	9.68±0.30	2.44±0.08	2.48±0.08	4.45±0.14	44.48±1.38

Our results of fatty acid profile are in accordance with the findings of *Zielinska et al. (2015)*, who compiled the nutritional composition of mealworm larvae and found content of oleic acid (40.86%) and linoleic acid (29.68%) as well as palmitic acid (18.0%). Similar results have also been found by *Mlcek et al. (2019)* for palmitic (18.6%) and oleic acid (36.9%), while the content of linoleic acid was much higher (30.9%). The contents of palmitic, linoleic and eicosanoic acid were in close agreement with previous report of *Gnosh et al. (2017)* that amounted to 4.71; 7.57 and 0.2 g/100 g DM, respectively.

In investigation of *Paul et al. (2017)*, the contents of oleic (35.83%), linoleic (22.83%) and γ-linolenic acid (0.11%) were considerably lower when compared to our results. Furthermore, the authors didn't find the presence of eicosanoic, docosanoic, eicosapentaenoic and docosahexaenoic acid. Their results were similar to research of *Ravzanaadii et al. (2012)* who reported of 0.05 g/100 g protein of γ-linoleic acid and found no Eicosapentaenoic and docosahexaenoic acid. This applies also for investigation of *Gnosh et al. (2017*) who reported only about 0.08 g/100 g DM of stearic acid. *Aguilar-Miranda et al. (2002)* also studied the fatty acid content, and they reported following values of fatty acid content (mg/100 mg of sample): palmitic (6.76); stearic (1.46), oleic (19.77) and eicosanoic acid (0.08). This and fatty acid content in the research from *Ravzanaadii et al. (2012)* were considerably higher when compared with our results (in g/100 g of protein) for palmitic acid (16.72), stearic acid (2.49), oleic acid (43.17), linoleic acid (30.23) and eicosanoic acid (0.24). *Paul et al. (2017)* also found higher values of palmitic acid (21.33) and stearic acid (7.92) in comparison with our results. The comparison between our results and other authors showed that considerable differences in the levels of fatty as well as amino acids in chemical composition of *T. molitor* larvae still existed. According to the earlier reports *(Ramos-Elorduy et al., 2002; Wyss, 2012; Finke and Oonincx, 2013; Broekhoven et al., 2015)* the most important reason for this could be feeding insects with different substrates. In this context, there are differences in the feeding regime of the above-mentioned authors. *Aguilar-Miranda et al. (2002)* reported that the larvae were grown on a sawdust bed, containing oat and corn flakes, dry bread, and pieces of vegetables as water supplement. In the research of *Bednarova et al. (2013*) all larvae and nymphs were fed a combination of wheat bran and carrots in the ratio 3:1 (w/w) for a 14-day period. *Gnosh et al. (2017)* raised mealworms on wheat bran with few leaves of Chinese cabbage as a source for water uptake and being nocturnal were kept in a dark environment. *Ravzanaadii et al. (2012)* stated that wheat bran was the main food for mealworm and vegetables such as cabbage, reddish and carrots were added as water source twice a week. In the investigation of *Siemianowska et al. (2013)* insects were fed oat flakes with addition of vegetables as a source of water. In the experiment of *Paul et al. (2017) T. molitor* larvae were reared on a diet containing wheat flour, wheat bran and brewer's yeast.

It can be seen from Table 2 that essential amino acids are highly represented in our samples. This primarily refers to isoleucine, tyrosine, phenylalanine, leucine, lysine and methionine.

Our results are similar to the amino acid profile published earlier by *Bednarova et al. (2013).* They reported that protein contain the following amounts of amino acids on dry weight: glutamate (6.51%), alanine (3.04%) and lysine (3.04%). *Zielinska et al. (2015)* observed a similar trend in the amino acid composition during their investigations on *T. molitor*. They reported that protein

contained (mg/g of protein) 26.1, 45.8 and 43.4 of threonine, leucine, and proline, respectively.

Table 2. The amino acids composition in mealworm

Amino acids	% DM or g/100 g DM	g/100 g protein	mg/g protein
ASP	4.30	7.71	77.13
GLU	6.44	11.54	115.44
SER	2.38	4.28	42.79
GLY	3.67	6.58	65.87
THR	1.47	2.63	26.36
ARG	3.60	6.45	64.54
ALA	4.53	8.11	81.14
TYR	3.86	6.92	69.28
VAL	0.65	1.17	11.76
MET	1.76	3.16	31.61
PHE	3.06	5.48	54.87
ILE	4.12	7.38	73.88
LEU	2.96	5.31	53.12
LYS	2.67	4.79	47.94
PRO	2.67	4.79	47.94

Abbreviations: Asp = Aspartate; Glu = Glutamate; Ser = Serine; Gly = Glycine; Thr = Threonine; Arg = Arginine; Ala = Alanine; Tyr = Tyrosine; Val = Valine; Met = Methionine; Phe = Phenylalanine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Pro = Proline

Furthermore, we found it important to compare our results with literature data that showed dissimilar findings when compared to ours. For this reason, we needed to recalculate our results into different units found in literature (Table 2). A good example for this is the publication of *Ravzanaadi et al. (2012)* with results which almost all (except valine) were considerably less than our findings (in g/100

g of protein): aspartate (3.59), glutamate (5.67), serine (2.09), glycine (2.41), threonine (1.80), arginine (2.43), alanine (3.68), tyrosine (3.46), methionine (0.67), phenylalanine (1.75), isoleucine (3.55), leucine (3.40), lysine (2.90) and proline (3.01). In the research of *Bednarova et al. (2013)*, they obtained the results of following content of amino acids (dry matter basis) arginine (3.03%), methionine (0.99%), phenylalanine (0.89%) and isoleucine (3.03%). The amino acid content was less when compared with our results. It is evident from table 2 that protein from the *T. molitor* larvae contained much higher levels of amino acids when compared with results in investigation of *Zielinska et al. (2015)*. They reported the following amounts (mg/g of protein) glutamate (79.7), glycine (31.8), arginine (25.6), alanine (44.3), tyrosine (28.8), methionine (9.6), phenylalanine (16.1), isoleucine (21.4) and lysine (26.7). *Gnosh et al. (2017)* studied the amino acid profile of *T. molitor* and reported that protein contained 2.76% aspartate, 5.78% glutamate, 2.20% serine, 2.61% glycine, 2.23% arginine, 3.96% alanine, 1.76% phenylalanine, 1.98% isoleucine, 2.01% lysine and 1.66% proline (dry matter basis). Their results also showed considerably lower amino acid content when compared to our study.

In contrast to previous comparisons, some of the above-mentioned authors obtained the results of amino acid content which were considerably higher than our results. *Bednarova et al. (2013)* published the following results (% DM): aspartate (4.66), serine (2.83), glycine (5.07), threonine (2.30), alanine (5.01), valine (3.42), leucine (5.47) and proline (3.92). Similar results were also reported from *Gnosh et al. (2017)*, threonine (1.83), tyrosine (3.45), valine (2.94) and leucine (3.37) in g/100 g DM. Furthermore, the report of *Janssen et al. (2017)* who found the following results (in g/100g of protein): aspartate (9.21), serine (5.03), threonine (4.52), valine (6.42), leucine (8.33), lysine (6.14) and proline (7.96).

Conclusion

In addition to the fact that *T. molitor* is a very rich source of protein and fat, our results showed a very good fatty acid and amino acid profile. Furthermore, high levels of essential fatty acids (linoleic), as well as essential amino acids (lysine, methionine, and threonine) make this a nutrient of high biological value. The differences in essential fatty and amino acid content between our results and discussed literature data, could be the consequence of different substrates used for rearing of insects. By substrate change, protein, and fat content, as well as amino and fatty acid profile could be modified, which we proved in our unpublished research. After everything stated above, the biological value of *T. molitor* larvae proves that it could be suitable as animal feed.

Masnokiselinski i aminokiselinski profil larvi crva brašnara (*Tenebrio molitor* L.)

Igor Jajić, Aleksandra Popović, Miroslav I. Urošević, Saša Krstović, Miloš Petrović, Darko Guljaš, Miljan Samardžić

Rezime

Crv brašnar (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) je jestivi insekt, rasprostranjen po celom svetu i pogodan za proizvodnju na industrijskom nivou. Brašnar se može koristiti i u komercijalne svrhe kao zamena za konvencionalne izvore proteina. U našem prethodnom istraživanju utvrđeno je da se suva materija larvi *T. molitor* pretežno sastoji od sirovih proteina (55,83%) i sirovih masti (25,19%), kao i malog sadržaja bezazotnih ekstraktivnih materija. Crvi su gajeni u inkubatoru pod kontrolisanim uslovima u plastičnim kutijama. Insekti su prosejani, a zatim stavljeni u posudu sa ključalom vodom i kuvani 180 sekundi. Sadržaj vlage je određen kao gubitak težine nakon sušenja larvi. Amino kiseline su analizirane na HPLC sistemu "Agilent Technologies 1260 series". Sadržaj masnih kiselina je utvrđen pomoću gasnog hromatografa "Thermo Scientific TRACE 1300" opremljenog sa plamenim jonizujućim detektorom korišćenjem "TR-FAME" kolona. Rezultati su pokazali visoki sadržaj nezasićenih masnih kiselina npr. oleinske kiseline (C18:1) koja čini većinsku lipidnu komponentu od 40,83%, za kojom sledi linolna kiselina (C18:2, omega-6 masna kiselina) sa 29,80% i linoleinska kiselina (C18:3) sa 1,08%. Esencijalne aminokiseline su visoko zastupljene u uzorcima (u % suve materije). To se pre svega odnosi na izoleucin (4,12), tirozin (3,86), fenilalanin (3,06), leucin (2,96), lizin (2,67) i metionin (1,76). Razlike koje su konstatovane u sadržaju esencijalnih masnih i amino kiselina u našim rezultatima u odnosu na analizirane literaturne podatke mogle bi biti posledica upotrebe različitih hraniva za gajenje insekata. Na osnovu svega navedenog, pokazalo se da bi larve *T. molitor* mogle biti pogodne kao hrana za životinje zbog svoje visoke biološke vrednosti.

Ključne reči: jestivi insekti, *Tenebridae*, hemijski sastav

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